PREDICTORS OF ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR TREATMENT RESPONSE IN NEOVASCULAR AGE-RELATED MACULAR DEGENERATION

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DECLARATION

This is to certify that:

1. this thesis comprises only my original work towards the Doctorate of Medical Science, except where noted in the Author Contribution

2. due acknowledgement has been made I the text to all other material used

3. the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices

Signed……..

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Predictors of Anti-Vascular Endothelial Growth Factor Treatment Response in Neovascular Age-Related Macular Degeneration

PREFACE

This thesis describes the candidate's part time work on a project at the Royal Victorian Eye and Ear Hospital and Centre for Eye Research Australia, looking at the predictors of outcome following the use anti vascular endothelial growth factor agents in the treatment of neovascular age related macular degeneration.

The work from this thesis is part of the Predictors of outcome in age related macular degeneration study, which is an on-going project that is led by Professor Robyn Guymer (Candidate's supervisor) looking at various factors that lead to suboptimal outcome in the treatment of neovascular age related macular degeneration, such as individual genetic make up and baseline clinical features.

Predictors of Anti-Vascular Endothelial Growth Factor Treatment Response in Neovascular Age-Related Macular Degeneration

ABSTRACT

Aim: Age related macular degeneration (AMD) is the commonest cause of legal blindness in individuals over 50 years of age. Although neovascular AMD only makes up around 10% of the cases of AMD, it contributes to about 90% of the cases of severe vision loss. The treatment of neovascular AMD has been revolutionised by the introduction of anti vascular endothelial growth factor (anti-VEGF) agents. With regular treatment, most patients can maintain vision and around one third of patients can have significant improvements in acuity.

However, despite timely and adequate treatment, approximately 10% of treated eyes continue to lose vision. The underlying reasons for this variable response have not been fully elucidated, although underlying patient and treatment factors have been suggested.

The aim of this work was to investigate factors that could potentially influence the efficacy of anti-VEGF agents, for improving vision in patients with neovascular AMD.

Methods: All patients with subfoveal/ juxtafoveal choroidal neovascularisation, secondary to AMD were recruited prospectively between 2006 and 2009, as part of an ongoing project looking at the predictors of outcome in patients with neovascular AMD undergoing anti-VEGF treatment. Different study questions made use of the available cohort in the retinal clinic at the time of the study initiation. As a result,

different numbers make up each cohort. Some participants may have been included in one or more studies but all were followed prospectively in the retinal clinics.

Anti-VEGF treatments administered compromised both ranibizumab and bevacizumab and in all cases three, monthly loading dose injections were performed. In some patients an, 'as required' dosing strategy was utilised thereafter, using strict re-treatment criteria, whilst in others a 'treat and extend' strategy was employed. All patients had optical coherence tomography (OCT) imaging at each visit, as well as colour fundus photography. A blood sample obtained after enrolment into the study, for genetic analysis. Baseline visual acuity was corrected for in all the studies of predictors of treatment outcome when using change in vision as an outcome measure.

Results: In a cohort of 185 patients, at six months from treatment initiation, a delay in treatment initiation from the first symptoms of neovascular AMD significantly impaired the likelihood of visual gain following anti-VEGF injections. A longer delay from symptom onset to the first injection was a significant predictor ($p=0.015$) of poorer treatment outcome, after controlling for age, sex, and baseline visual acuity. Patients with a delay in treatment of 21 weeks or more had a 2.62 (1.20, 5.68) greater chance of worsening vision after treatment compared to those with a delay of 7 weeks or less.

Qualitative changes on time domain OCT imaging were also a predictor of visual outcome after 12 months of anti-VEGF treatment. In a cohort of 214 eyes of 144 patients receiving anti-VEGF injections, the presence of intraretinal cystic oedema on OCT at baseline was associated with worse visual outcome at 12 months, compared to where only subretinal fluid (SRF) was present. Similarly, the failure to clear intraretinal fluid (IRF) from the macula, following three, monthly anti-VEGF injections, was predictive of a six fold greater chance of vision loss at 12 months, compared to

where IRF was cleared.

Significant hyper-reflectance at the level of the RPE was also strongly associated with worse visual outcomes regardless of whether there was associated SRF or IRF. Most of the eyes with this feature had significant subretinal haemorrhage at baseline and went on to develop subretinal fibrosis and/ or atrophy through the course of treatment. Thus this feature, when present predicted a worse outcome for vision, even when IRF or SRF was absent.

Several genetic polymorphisms coding for proteins involved in the extracellular matrix composition, vascular endothelial growth factor production and lipid metabolism were also predictive of a poorer outcome to anti-VEGF therapy.

In 224 consecutive patients with neovascular AMD, treated with anti-VEGF agents in an 'as required' regime, the AA (homozygote risk) genotype at rs11200638 - *HTRA1* promoter single nucleotide polymorphism (SNP), $p = 0.001$ and GG (homozygote risk) genotype at rs10490924 (A69S) in *LOC387715/ARMS2* ($p = 0.002$) were each significantly associated with poorer visual outcome at 12 months. SNPs associated with the complement pathway genes were not significantly associated with visual outcome.

When a cohort of 201 patients were investigated for the influence of 7 SNPs within the *VEGF* gene and the outcome of anti-VEGF treatment, we demonstrated that variants of the SNP at position rs3025000 of the *VEGF* gene were associated with vision outcome at 6 months.

The presence of the T allele (TC or TT genotypes) at this SNP predicted a better outcome of +7 letters at 6 months compared with the CC genotype. In a subgroup analysis, presence of the T allele predicted a significantly higher chance of the patients belonging to the good responder group (gain of ≥5 letters from baseline) after 3, 6, and 12 months treatment.

The E4 allele of the *APOE* gene was associated with an improved treatment outcome. In a cohort of 192 patients we found that the presence of the E4 allele was associated with an improved treatment outcome at 3 ($p = 0.02$) and 12 ($p = 0.06$) months, compared with the presence of the E2 allele. Subjects with an E4 allele had a four times greater chance of a 2-line gain in vision at 3 months.

Other anatomical factors were also predictive of vision change. Larger retinal venular calibre at baseline was also associated with poorer response to treatment. In a group of 88 patients, we found that after accounting for patients age, gender, size of choroidal neovascularization and number of injections, patients who lost vision from baseline to 12 months, had significantly larger baseline central retinal vein equivalent (CRVE) compared with those who were stable and those who improved, $p = 0.007$).

Finally we reviewed our treatment patterns and outcomes over the years since 2006 when anti-VEGF treatment commenced in our retinal clinics. In comparison to the pivotal trials, in an audit of our first year's experience with anti-VEGF injections we found a change in acuity of +4.0 ± 11.9, +1.6 ± 12.6, -3.4 ± 15.5 and -6.9 ± 17.8 letters at 3, 6, 9 and 12 months respectively. Only a mean number of 4.2 ± 2.0 injections were administered in the first 12 months, the low number reflecting a trial of treatment in many chronic cases being seen in the clinic when treatment became available. More recently, with the use of a 'Treat and Extend regime', we demonstrated a mean improvement of +9.5 (10.9) letters at 12 months from baseline. In 67 patients who completed 24 months follow-up, the mean change in VA was +8.2 (12.3) letters. These results were achieved with a mean of 8.6 (1.0) and 5.9 (2.3) over the first and second years of treatment, respectively. The visual outcome over 24 months was very similar to those of the pivotal clinical trials with monthly injections, with significantly less injections.

With respect to complications of treatment, we retrospectively collected a series of patients presenting with inflammation following intravitreal anti-VEGF injections in order to determine if there were any commonly associated features and whether inflammation affected final visual outcome. Over a 12 month period, 19 cases of severe inflammation were observed out of 1278 intravitreous injections of bevacizumab that were given (1.49%). Mostly the presentation was acute, with most patients presenting within a day of injection with rapid reduction in vision, but minimal discomfort. The visual acuity at presentation was substantially reduced compared to the pre-injection acuity, although the vision rapidly improved with treatment over a period of 7-25 days toward pre-injection visual acuity, suggesting that early recognition allowed appropriate management of the inflammation and thus the final vision was not compromised.

Conclusions: By determining factors which influence the visual acuity and anatomic outcomes after anti VEGF treatment, it is hoped that we can begin to optimise and personalise an approach to dosing individual patients so that every patient has the greatest chance of benefiting from these treatments which in the past decade have halved the rate of blindness from this common threat to vision.

ACKNOWLEDGEMENTS

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Last, but not least, I thank my family for their patience and support.

PUBLICATIONS RELATING TO THIS THESIS

Much of the work in this thesis has a collaborative element. The research team into the predictors of outcome in patients with neovascular AMD was led by Professor Robyn Guymer and she is the senior author on most publications.

The full copy of the peer-reviewed publications that resulted from this body of work is collated in the appendix section of this thesis.

In the majority of publications I was the first author, where I initiated, designed and recruited patients into a project looking at factors that affect the outcome of anti vascular endothelial growth factor treatment for neovascular age related macular degeneration. In these publications, I wrote the majority of the text, although significant input was given by my co-authors.

1. Predictors of AMD treatment response.

Wickremasinghe SS, Sandhu SS, Busija L, Lim J, Chauhan DS, Guymer RH. Ophthalmology. 2012 Nov;119(11):2413-2414.

2. Retinal venular caliber predicts visual outcome after intravitreal ranibizumab injection treatments for neovascular AMD.

Wickremasinghe SS, Busija L, Guymer RH, Wong TY, Qureshi S. Invest Ophthalmol Vis Sci. 2012 Jan 3;53(1):37-41.

3. Lessons learnt inform our approach to new antivascular endothelial growth factor treatments for neovascular age-related macular degeneration.

Wickremasinghe S, Abedi F, Sandhu SS, Guymer R. Clin Experiment Ophthalmol. 2013 Nov;41(8):723-6.

4. Variants in the APOE gene are associated with improved outcome after anti-VEGF treatment for neovascular AMD.

Wickremasinghe SS, Xie J, Lim J, Chauhan DS, Robman L, Richardson AJ, Hageman G, Baird PN, Guymer R. Invest Ophthalmol Vis Sci. 2011 Jun 8;52(7):4072-9.

5. Acute intraocular inflammation after intravitreous injections of bevacizumab for treatment of neovascular age-related macular degeneration.

Wickremasinghe SS, Michalova K, Gilhotra J, Guymer RH, Harper CA, Wong TY, Qureshi S. Ophthalmology. 2008 Nov;115(11):1911-5

I was a major contributing author in the following papers.

6. Delay to treatment and visual outcomes in patients treated with anti-vascular endothelial growth factor for age-related macular degeneration.

Lim JH, **Wickremasinghe SS**, Xie J, Chauhan DS, Baird PN, Robman LD, Hageman G, Guymer RH. Am J Ophthalmol. 2012 Apr;153(4):678-86

7. Genetic influences on the outcome of anti-vascular endothelial growth factor treatment in neovascular age-related macular degeneration.

Abedi F, **Wickremasinghe S**, Richardson AJ, Islam AF, Guymer RH, Baird PN. Ophthalmology. 2013 Aug;120(8):1641-8.

8. Variants in the VEGFA gene and treatment outcome after anti-VEGF treatment for neovascular age-related macular degeneration.

Abedi F, **Wickremasinghe S**, Richardson AJ, Makalic E, Schmidt DF, Sandhu SS, Baird PN, Guymer RH. Ophthalmology. 2013 Jan;120(1):115-21.

9. Anti-VEGF treatment in neovascular age related macular degeneration. A Treat-and-Extend Protocol Over 2 Years

Abedi F, **Wickremasinghe SS**, Islam FA, Guymer RH. Retina 2014; 34: 1531-38.

10. Ranibizumab treatment for neovascular age-related macular degeneration: from randomized trials to clinical practice.

Michalova K, **Wickremasinghe SS**, Tan TH, Chang A, Harper CA, Downie JA, Hunyor AP, Guymer RH. Eye (Lond). 2009 Aug;23(8):1633-40.

11. Predictors of anti-VEGF treatment response in neovascular age-related macular degeneration.

Finger RP, **Wickremasinghe SS**, Baird PN, Guymer RH. Surv Ophthalmol. 2014;59(1):1-18.

ABBREVIATIONS

AMD: Age related macular degeneration

ANCHOR: *AN*ti-VEGF antibody for the treatment of predominantly classic *CHOR*oidal neovascularization in age-related macular degeneration

APOE: Apoliporotein E

AREDS: Age-related Eye Disease Study

ARMS2: Age related maculopathy susceptibility 2

BCVA: Best corrected visual acuity

CATT: *C*omparison of *A*ge-Related Macular Degeneration *T*reatments *T*rials)

BF: Complement factor B

CFH: Complement factor H

CFHR: Complement factor H receptor

CSCR: Central serous chorioretinopathy

C2: Complement component 2

C3: Complement component 3

C5: Complement component 5

CNV: Choroidal neovascularization

CRT: Central retinal thickness

ETDRS: Early treatment for diabetic retinopathy study

FFA: Fundus fluorescein angiography

HTRA1: High temperature requirement A-1

IVAN: *I*nhibit *V*EGF in *A*ge-related choroidal *N*eovascularisation

IRF: Intraretinal fluid

LogMAR: Logarithm of the minimum angle of resolution

LUCAS: *LU*centis *C*ompared to *A*vastin *S*tudy

MARINA: *M*inimally classic/occult trial of the *A*nti-VEGF antibody *R*anibizumab *I*n the treatment of *N*eovascular *A*ge-related macular degeneration

OCT: Optical coherence tomography

PIER: *E*fficacy and Safety of *R*anibizumab in Subjects with Subfoveal Choroidal CNV Secondary to AMD

PDT: Photodynamic therapy

PED: Pigment epithelial detachment

PlGF: Placental growth factor

PRN: Pro re nata

PrONTO: *Pr*ospective *O*CT Imaging of Patients with *n*AMD *T*reated with intra*o*cular Ranibizumab

RAP: Retinal angiomatous proliferation

RPE: Retina Pigment epithelium

SRF: Subretinal fluid

tSNP: Tagged single nucleotide polymorphism

VA: Visual acuity

VEGF: Vascular endothelial growth factor

VIEW: *V*EGF Trap-Eye: *I*nvestigation of *E*fficacy and Safety in *W*et AMD

FIGURES

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CHAPTER ONE

Impact of age related macular degeneration, treatment options and current knowledge of influences on treatment outcomes: A brief review of the literature

AGE-RELATED MACULAR DEGENERATION

AMD is the most common cause of legal blindness in all industrialised countries, and affects a large proportion of the elderly.[1,2] Severe vision-loss occurs in the advanced stages of the disease, which are classified into neovascular or “wet” AMD and atrophic or “dry” AMD, both stage 4 disease, according to the AREDS severity classification scale.[3]

Visual loss secondary to AMD increases exponentially with age, such that the prevalence is around 1% in those under 65 years and about 25% in those over 90 years[4] **(Figure 1)**.

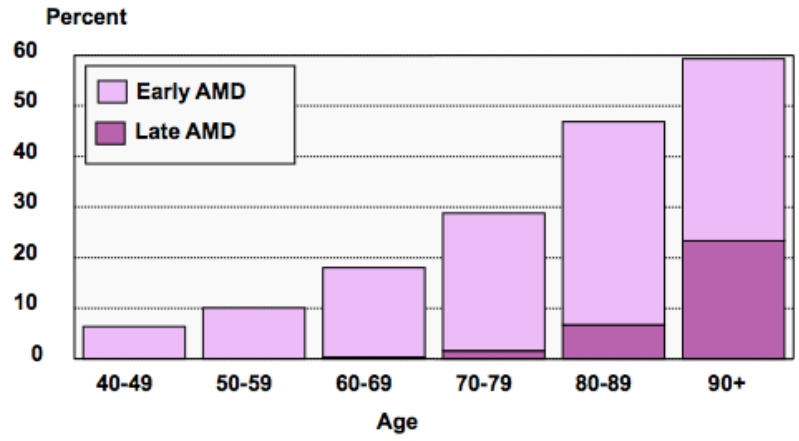


Figure 1. Prevalence of early and late age-related macular degeneration, by age group (Melbourne Vision Impairment Project, 2001)

With the changing demographics in Western societies, the prevalence of AMD is expected to double in the next 20 years and it has been estimated that 2.95 million

people in the United States will have advanced AMD by 2020.[5] This has a long-term impact on both the individuals and families of those affected, but also places a massive burden on already strained health resources. Although neovascular AMD comprises around 10-20% of all cases of AMD, it accounts for 80-90% of blindness caused by AMD.

The aetiology of AMD is still not fully characterised, although it is generally considered to be a complex genetic disorder where environmental exposures interact with a pre-determined genetic risk. Over the past decade several groups have described specific genetic polymorphisms that have shown very significant associations with AMD. Most notably, genes associated with the pathway of the complement cascade, Complement factor H (CFH)[6-8], the complement factor B (*BF*)[9], complement component 2 (*C2*) genes,[9] the complement *C3* gene,[10] and the *CFH* paralogous genes (*CFHR3, CFHR1, CFHR4, CFHR2* and *CFHR5*) have also been implicated.[11] The complement system is part of the innate immune system and eliminates bacterial and viral infections. It may also trigger an inflammatory response and remove debris from cells and tissues. This system must be carefully regulated to avoid inappropriate activation and subsequent damage to healthy cells.

Other genes that are not involved in inflammation have also been implicated. Variation in the *HTRA1 and LOC387715ARMS2* locus on chromosome 10 has been convincingly associated with AMD, with an effect size similar to or greater than that seen with *CFH*.[12-14] These genes are thought to be involved in extracellular matrix deposition/ breakdown and have been shown to confer a greater risk for wet AMD development than for geographic atrophy.[15]

The *APOE* gene has also been identified. This gene codes for a protein involved in lipid metabolism. Several epidemiologic studies in different populations of AMD[16-19]

have shown associations between the APOE polymorphisms and risk of developing AMD.

Recently a genome wide association study assessing >17,100 advanced AMD cases and >60,000 controls of European and Asian ancestry, identified a further seven loci near the genes, COL8A1-FILIP1L, IER3-DDR1, SLC16A8, TGFBR1, RAD51B, ADAMTS9 and B3GALTL that were associated with a greater risk of AMD development. These loci showed enrichment for genes involved in the regulation of complement activity, lipid metabolism, extracellular matrix remodelling and angiogenesis.[20]

Certain lifestyle choices may also represent “at risk” behaviours and modification of these behaviours generally and especially in those with a family history of AMD and in those with early signs of the disease may become the focus of public health awareness programs that are aimed at preventing vision loss.

Smoking has consistently been identified as a risk factor for the development of advanced AMD. Other potential risk factors have been suggested, although the results of studies have not shown uniform results. Factors for which there is mounting evidence include high fat intake and obesity, alcohol and red meat intake. A protective role has also been hypothesized for those with high levels of oily fish and long chain polyunsaturated fatty acid intake and antioxidants.[21,22]

NEOVASCULAR AGE-RELATED MACULAR DEGENERATION

Recently there have been major advances made in the treatment of neovascular AMD **(Figure 2)**. Currently there is however, very little to offer the great majority of people with the atrophic form of advanced AMD, or early and intermediate AMD, apart from lifestyle changes and ensuring a healthy diet, and in some cases, the use of vitamin supplementation.[21,22]



Figure 2. Colour fundus and late phase fluorescence angiogram of the left eye in a patient with neovascular age- related macular degeneration.

Neovascular AMD is characterized by the growth of choroidal blood vessels through Bruch's membrane into the subretinal RPE space, usually leading to accumulation of fluid in the sub-RPE space and detachment of the RPE.[23] Fluid may also accumulate in the subretinal space as well as the intraretinal compartment. The underlying aetiology of this pathological growth of choroidal blood vessels through Bruch's membrane, is thought to be a result of over-expression of vascular endothelial growth factor-A (VEGF-A; also referred to as VEGF),[24] which induces endothelial cell proliferation, migration, and survival.[25]

VEGF-A is a member of the VEGF family of growth factors that also includes VEGF-B, VEGF-C, VEGF-D and PlGF. Alternative RNA splicing of the human VEGF-A gene results in the formation of four major isoforms (VEGF121, VEGF165, VEGF189, and VEGF206) and at least five minor isoforms (VEGF145 , VEGF148 ,VEGF162 , VEGF165b , andVEGF183).[26] VEGF165 and VEGF121 have been suggested to have the strongest angiogenic potential.[27] In addition to promoting angiogenesis, VEGF-A also affects vascular permeability by inducing formation of pores in the vascular endothelial cells and by disrupting the intercellular junction between these cells.[28] In neovascular ocular diseases, the oedema from new, permeable blood vessels can disrupt the retinal anatomy and separate the retina from underlying structures, potentially causing severe vision loss.

Until recently the main treatment strategies from neovascular AMD were aimed at ablating the abnormal choroidal neovascular complex. These treatments included thermal laser photocoagulation and PDT. Thermal laser photocoagulation, with blue-green or green argon laser of sufficient intensity to produce nearly white lesions in the retina and cover the entire neovascular lesion, as well as adjoining blood, reduced the risk of additional and severe loss of vision, when compared to natural progression of the disease, with a doubling of the chance of maintaining or increasing acuity compare to observation. Despite this, laser was associated with a 48% chance of ≥6 lines vision loss and a 54% chance of recurrent disease at 5 years.[29] PDT was also used to ablate the underlying neovascular complex, but this treatment utilised the photosensitive properties of verteporfin. This dye accumulates in the abnormal blood vessels and, when stimulated by non-thermal red light (wavelength of 693 nm), in the presence of oxygen, produces highly reactive short-lived singlet oxygen and other reactive oxygen radicals, resulting in local damage to the endothelium and blockage of the vessels. Unfortunately, improvement in vision

was uncommon and the goal of treatment was to prevent the loss of ≥3 lines of acuity (>15 letters on the early treatment for diabetic retinopathy study chart).[30]

Both thermal laser and PDT were inherently destructive to the RPE and the outer retina and thus permanent scotomas were often visible.

Fortunately the treatment of neovascular AMD has been revolutionised over the last decade, with the introduction of intravitreal injections of anti vascular endothelial growth factor agents. Pegaptanib, a pegylated aptamer against VEGF (Macugen, Eyetech/ Pfizer) was the first agent which showed promise in stabilising progression of vision loss in neovacular AMD,[31] although it was not until the pivotal randomized clinical trials, ANCHOR (*AN*ti-VEGF antibody for the treatment of predominantly classic *CHOR*oidal neovascularization in age-related macular degeneration)[32] and MARINA (*M*inimally classic/occult trial of the *A*nti-VEGF antibody *R*anibizumab *I*n the treatment of *N*eovascular *A*ge-related macular degeneration),[33] identified that Ranibizumab, a monoclonal antibody against VEGF (Lucentis, Genentech/Novartis) was effective at arresting progression and actually improved vision in some patients. These trials demonstrated that, with monthly injections of ranibizumab, around 1/3 of patients with subfoveal choroidal neovascular AMD, improve 15 letters of vision, with a mean gain of +11.3 and +7.2 letters at 12 months and +10.7 letters and +6.6 letters at 24 months, in predominantly classic and minimally classic/ occult lesions respectively. This compares with a loss of -9.8 letters with PDT in the ANCHOR trial and a loss of -14.9 letters in the natural history group in the MARINA study.

Similarly a vision loss of ≥ 15 letters, with ranibizumab treatment was prevented in around 90% of individuals, in both studies, after 2 years of treatment (Figure 3).

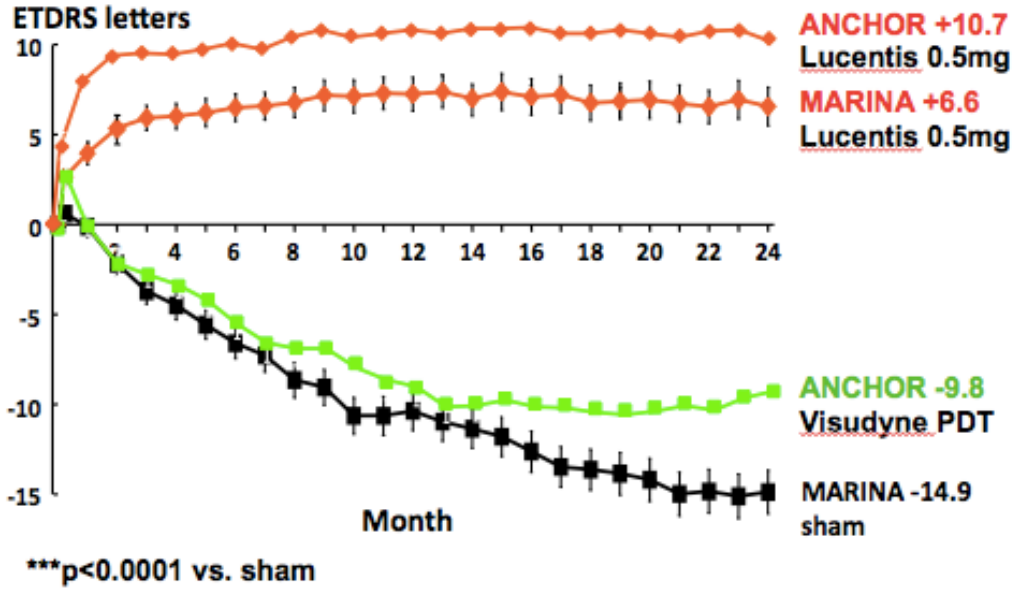


Figure 3. Change in vision over time following ranibizumab injections in the ANCHOR and MARINA trials.

Bevacizumab (Avastin, Genentech) is another anti-VEGF monoclonal antibody. Bevacizumab has been widely used in an “off label” treatment for neovascular AMD because it is substantially cheaper than ranibizumab. The recent CATT and IVAN studies have shown that ranibizumab and bevacizumab have similar effects on VA if administered under the same treatment protocol. These studies also demonstrated that variable dosing of the two agents, based on optical coherence tomography detection of macular fluid, was ‘non-inferior’ to monthly injections, after 1-2 years.[33,34] The results of the CATT trial are shown in Figure 4.

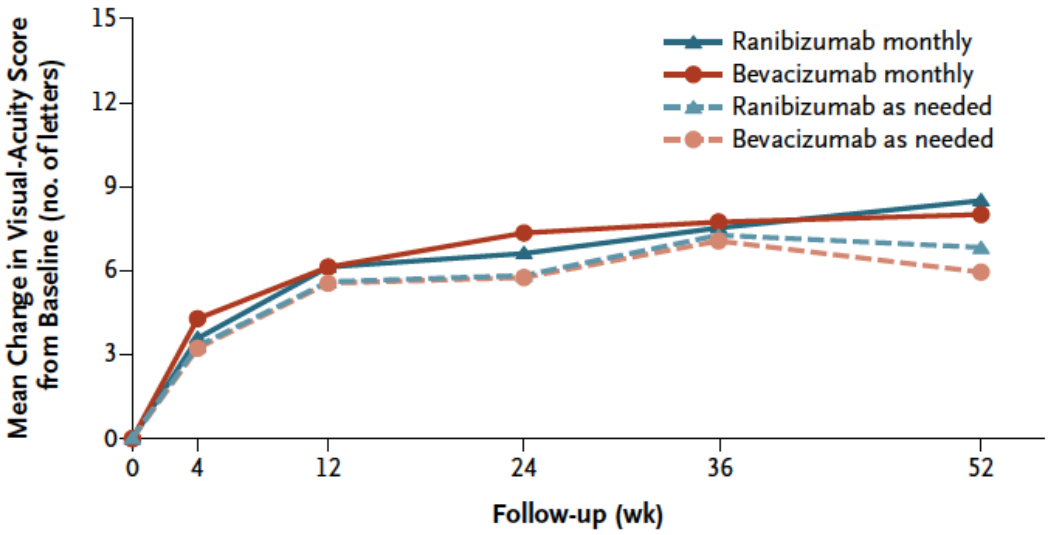


Figure 4. Comparison of mean vision changes between ranibizumab and bevacizumab in the CATT trial.

Recently, aflibercept (Eylea, Regeneron/ Bayer) has also been approved for use in the treatment of neovascular AMD. The multi-centre VIEW 1 and 2 studies demonstrated equivalence of 3 doses of aflibercept and the commercially available (0.5mg/0.05mls) dose of ranibizumab. All strategies result in around +8 letters gain in vision, with approximately 1/3 improving by 15 letters (Figure 5). The 2.0mg dose of aflibercept, given every other month (after 3 initial monthly injections), appeared to have similar vision results to monthly ranibizumab treatment (Figure 5). Unfortunately, a 2 monthly ranibizumab treatment arm was not assessed for meaningful comparison. It is however encouraging that bi-monthly treatment with aflibercept is, on average, successful at treating patients with neovascular AMD.[35]

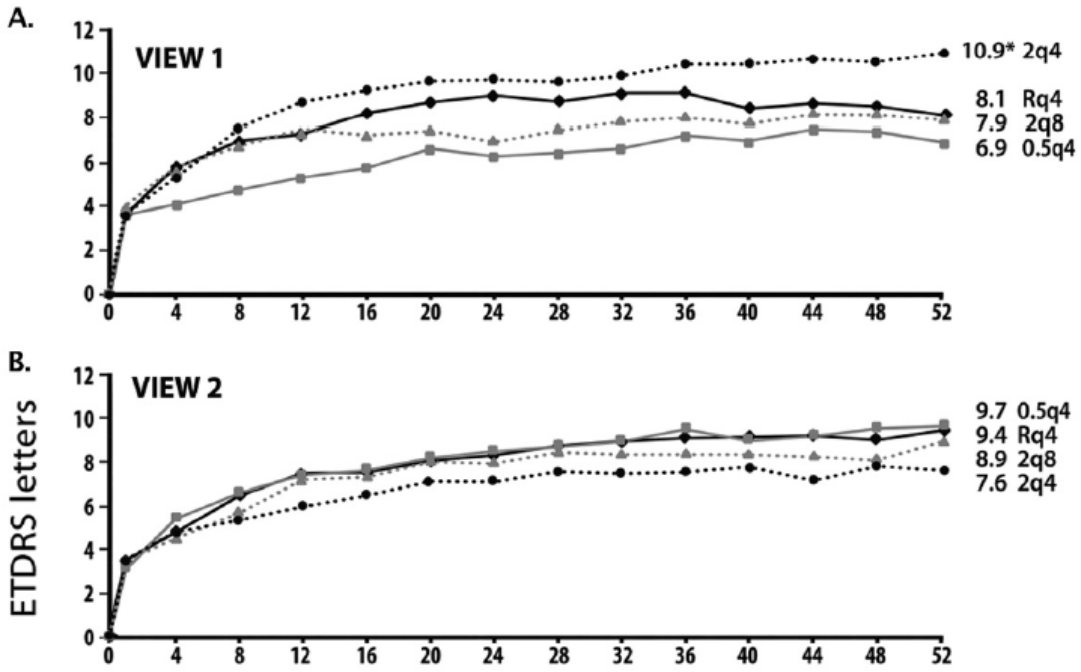


Figure 5. Mean change in vision after treatment with aflibercept (3 doses) and ranibizumab at 12 months.

In all studies of anti-VEGF treatment for AMD however, there is still a significant percentage of the treated patients (approximately 10%) who continue to lose vision despite treatment.[32,33]

It has also been demonstrated that not all patients require monthly anti-VEGF dosing to achieve optimal visual results. The PIER trial[36] utilized a quarterly dosing regime following three, monthly loading dose injections of ranibizumab. Although the treated group had better vision at 12 months, compared to the sham-treated patients, the initial gains in visual acuity observed over the loading dose period were lost and the vision at 12 months was similar to baseline. It was noted that not all patients treated with this regime fared equally, with some patients maintaining vision gains that were achieved during the monthly dosing period and others either not responding at all, or

initially responded well, then failed to maintain the initial gains. The SUSTAIN trial[37] provided further evidence of this differential response to treatment. This trial examined ranibizumab treatment on a PRN basis, following administration of three initial loading dose injections. Re-treatment was based on specific visual acuity, clinical and optical coherence tomography criteria. In these patients, approximately 50% maintained their initial sharp gains in visual acuity, the so-called “gain and maintain” group. Another third of patients enjoyed an initial gain in visual acuity, but failed to maintain this gain during follow-up, the “gain and not maintained group”. Most of the acuity increase, in these two groups, occurred in the first three months of treatment. Approximately 25% of patients never experienced any gain in vision, the “no initial gain” group. In these patients, vision failed to improve from the outset, with continued losses being noted during the course of treatment regimen. Overall, around 20% of patients did not require any further treatment following the initial loading doses to maintain acuity. (Figure 6)

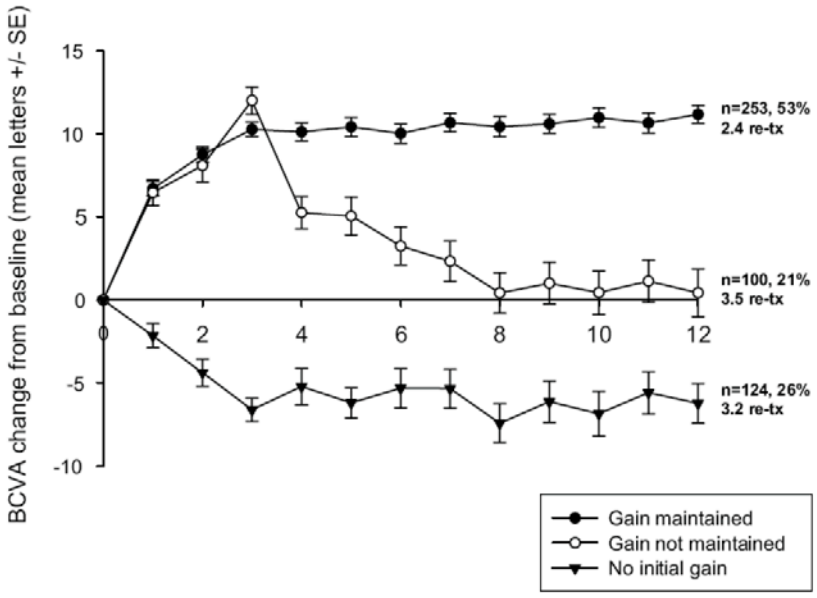


Figure 6. The different types of responder with the variable dosing regime utilised in the SUSTAIN trial.

At present, we do not fully understand what factors dictate this differential response but variables such as baseline visual acuity, lesion size, delay to treatment and age do appear to influence outcomes of anti-VEGF therapy.[38-40] The potential role of genetic variation in determining outcome has also been investigated.

Understanding the important determinants of treatment outcome would allow us to start to address them so that a greater number of patients could reap the maximum benefits from this and potentially other potential anti-VEGF treatments.

Additionally anti-VEGF treatment for neovascular AMD is resource intensive. Knowledge of likely treatment response will allow a personalised treatment strategy. This will potentially minimise treatment frequency in many patients and predict when alternative treatment regimes may be useful, in those patients with a potential for poor response with anti-VEGF therapy.

CURRENT KNOWLEDGE OF FACTORS ASSOCIATED WITH POOR OUTCOME WITH ANTI-VEGF TREATMENT

The ANCHOR and MARINA subgroup analyses, as well as other studies demonstrated have previously demonstrated that increasing age and neovascular lesion size are associated with poorer visual outcome following anti-VEGF treatment. Additionally, “under-treatment” as observed in the quarterly dosing regime within the PIER[36] and EXCITE[41] studies led to a failure to maintain visual gains in many patients.

Better baseline visual acuity has also been associated with increased risk of vision loss, with eyes with poorer vision more likely to improve. This is probably explained by the so called “floor” and “ceiling” effect, where eyes with good vision are unable to

improve substantially and those with very poor vision, being unable to deteriorate further.[38,39,42]

CHARACTERISTICS OF CHOROIDAL NEOVASCULARISATION

Although increasing CNV size has been identified as a risk factor for poorer response to anti VEGF treatment,[38,39] the type of choroidal neovascular membrane, as determined by fundus fluorescein angiography is not established as a predictive factor. Some studies have suggested a differential response to anti-VEGF treatment, based on the baseline CNV characteristics. The studies are conflicting however, with predominantly (Figure 7) and minimally classic lesions being associated with less gain in vision[43] and more frequent recurrences compared to occult lesions [44] (Figure 8). Other studies however, have reported that classic CNVs (predominantly and/or minimally) gain more vision than occult lesions.[45] A further study reported that RAP, a type of occult CNV, is more likely to gain vision than other lesions.[43]

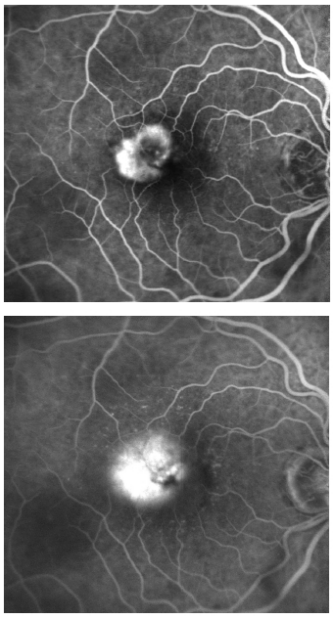


Figure 7. Early and late frames of fluorescein angiogram showing a subfoveal classic choroidal neovascular membrane.

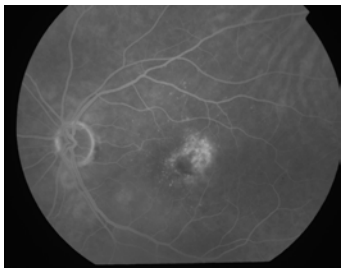


Figure 8. Late frame of fluorescein angiogram showing speckled hyperfluoresence from an occult

OPTICAL COHERENCE TOMOGRAPHY CHARACTERISTICS

Another imaging modality that is used to help diagnosis and aid management of neovascular AMD is OCT- Figure 9.

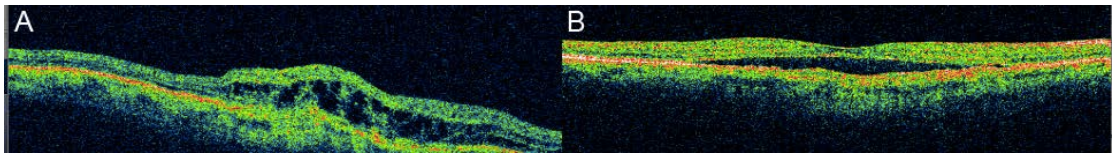


Figure 9. OCT appearance of (A) Intraretinal fluid and (B) Subretinal fluid.

IRF is usually contained in cystic spaces, and appears optically clear (black). This can occur in vascular occlusive diseases that cause macular oedema such as DR, or be early stages of neovascular AMD. SRF collects under the neural retina. In addition to neovascular AMD, SRF may be observed in conditions such as central serous chorioretinopathy, uveitis and retinal vascular disease. RPE detachments are generally seen in AMD as serous or fibrovascular in nature. They can be clear, but can also have some reflectivity if there is inflammation or blood.

In RPE atrophy, the presence of less pigment leads to reduced absorption of the incident light at this level, revealing more detail of the underlying tissue, the

choriocapillaris, with increased signal from this level. Very highly reflective (clinically dense) structures such as subretinal scars, hard exudates and blood vessels can attenuate the signal significantly to produce a “shadow” of absent reflectivity.

Several OCT characteristics have been associated with functional treatment outcomes. The presence of a foveal pit, absence of central subretinal fibrosis and an intact inner segment/outer segment photoreceptor layer have been shown to predict a greater chance of visual improvement following anti-VEGF treatment.[46] Interestingly, central retinal thickness (a global measure of macular anatomy), at baseline appears not to be related to functional outcomes, at least at six months. Conversely, increased foveal thickness and the presence of a retinal pigment epithelial detachment, have been associated with a decreased chance of gain in vision at 12 months.[43] In general however, OCT features are a much weaker predictor of visual acuity outcomes, compared to the baseline visual acuity.[46]

GENETIC FACTORS

The potential role of genetic variation in determining outcome has also been investigated. Whilst multiple studies have implicated roles for several genes including *CFH*, *HTRA1/ARMS2*, *C3*, *CFB/C2* and *APOE* in the risk of developing AMD,[47] there is no consensus in the literature regarding the influence of these genetic polymorphisms in the response to anti-VEGF treatment.

CFH gene

The *CFH* gene codes for a protein known as complement factor H. This protein helps

regulate the complement system. A number of studies have reported an association with variants of this gene and visual acuity outcomes following anti-VEGF treatment at 12 months[48,49] follow up, whilst others have failed to identify any association with treatment.[50] Within the studies that report an association, several have found the CC risk genotype of single nucleotide polymorphism (SNP) rs1061170 (Y402H) in the CFH gene to be associated with a less favourable outcome of treatment as well as a need for more injections during follow up.[49,51,52] Conversely, the TT protective genotype was reported in one study to be the least likely to gain vision (> 5 ETDRS letters).[53] Thus both risk and protective genotypes have been associated with worse outcome in these studies.

HTRA1/ARMS2 genes

HTRA1/ARMS2 are genes coding for proteins involved in extracellular matrix function. Several polymorphisms in and near the *ARMS2* gene have been associated with the development of AMD. The best studied of these variations is known as rs10490924. The *ARMS2* gene is in linkage disequilibrium with the *HTRA1* gene. Changes in this gene have also been studied as a risk factor for AMD. Thus, it is difficult to tell whether changes in one gene or the other, or possibly changes in both genes, account for the increased AMD risk. Homozygosity for the TT genotype at SNP rs10490924 (A69S) of the *ARMS2* gene has been reported to increase the risk of losing vision or not improving under treatment.[54] Similarly, the TT genotype has been reported to be associated with a lack of response to treatment[55] and the CC genotype with better treatment outcomes.

With respect to the HTRA1 gene, the results on treatment are not consistent, with studies finding positive, negative as well as no association with outcome. [49,50,55-58]

VEGF-A gene

Treatment outcome may also be affected by the extent of VEGF production. It is possible that certain variants of the *VEGF* gene may affect production of VEGF and thus affect the compromise the efficacy of anti-VEGF treatment to improve anatomical / visual outcomes.

The *VEGF* gene is a member of the platelet-derived growth factor (PDGF)/vascular endothelial growth factor (VEGF) family and encodes a protein that is a glycosylated mitogen that specifically acts on endothelial cells and has various effects, including mediating increased vascular permeability, inducing angiogenesis, vasculogenesis and endothelial cell growth, promoting cell migration, and inhibiting apoptosis. VEGF-A mediates the growth of new blood vessels from pre-existing vessels (angiogenesis) by binding to the cell surface receptors VEGFr1 and VEGFr2 located on endothelial cells.

To date, a number of studies have assessed the association between vascular *VEGF* gene and anti-VEGF treatment outcomes in neovascular AMD. Although the majority of studies have not found any association,[49,53,55,59] or only an association in univariate analyses, an American study reported an association with fewer injections[48] and a Korean and two Japanese[60,61] studies reported associations with BCVA results.

Apoliprotein E

The *APOE* gene has been also been linked with AMD. APOE is a class of apolipoprotein found in the chylomicron and Intermediate-density lipoprotein (IDLs) that is essential for the normal catabolism of triglyceride-rich lipoprotein constituents. It binds to receptors of the low-density lipoprotein receptor gene family.

Seddon et al found that the APOE E4 allele frequency in patients with exudative AMD was lower than in subjects without the disease (12.1% vs. 28.6%; p<0.0009).

The *APOE* gene is polymorphic with three major isoforms: *APOE E2, APOE E3* and *APOE E4*.[62] Although these allelic forms differ from each other by only one or two amino acids at positions 130 and 176, these differences alter the APOE structure and function. It has been postulated that APOE E4 allele may greater facilitate lipid efflux from RPE cells and lipid transport across Bruch's membrane, an effect secondary to the prevention of normal dimerization of the apolipoprotein E protein that usually occurs with the APOE E3 and APOE E2 isoforms. The influence of the *APOE* gene on the outcome of anti-VEGF has not been extensively investigated.

Other genes

Although several components involved in the alternate pathway of the complement system have been implicated in the development of AMD, several studies have failed to detect any association with polymorphisms in the C2, C3, C5, CFB or CFHR genes and treatment outcome.[49,55] Only one study reported C3 to be associated with reduced CRT on OCT following treatment.[48]

Multiple gene studies

The influence of multiple AMD- risk genes on anti-VEGF treatment has also been studied.

One such study assessed polymorphisms within the *CFH*, *ARMS2/HTRA1* and *VEGF* genes in relation to treatment outcomes and age at onset of treatment.[63]

An increasing number of risk alleles adversely affected treatment outcomes and the age at onset of treatment lower. Cases with all four high risk alleles and newly

identified risk alleles in the *VEGF* gene had a mean 20 letter loss from baseline to follow-up after upload and were on average ten years younger at onset of treatment (70 vs. 80 years) compared to cases with no risk alleles.[63]

Contrary to these findings, the recent CATT study found no association between polymorphisms in the *CFH*, *C3*, *ARMS2* and *HTRA* genes and response to anti-VEGF treatment in neovascular AMD, with respect to final vision, change in visual acuity from baseline, the degree of anatomic response (fluid on OCT or FA, retinal thickness, change in total foveal thickness, change in lesion size), or the number of injections after 12 months.[64]

RETINAL VASCULATURE

Retinal vascular calibre is an indirect indicator of ocular blood flow that can be measured from fundus photographs.[65] Changes in retinal vascular calibre have been linked with systemic and ocular diseases, and may reflect changes in retinal blood flow, inflammation, ischaemia and endothelial function.[66,67]

In systemic disease, retinal venular calibre changes have been shown to predict the development of incident cardiovascular diseases, such as stroke and coronary heart disease, independent of traditional cardiovascular risk factors.[68-71] It has also previously been shown that in early AMD, retinal venular diameter is independently associated with disease, with increased diameter seen in those with early disease compared to healthy individuals.[68] This suggests that AMD may have similar aetiological risk factors to cardiovascular disease, such as local inflammation, oxidative stress, and nitric oxide dysregulation.[72-74]

VEGF is widely expressed in the normal retina and as well as in various organs such

as the heart, lungs and kidneys, where several roles for the cytokine have been identified. Systemic administration of anti-VEGF agents has been linked to the development of complications such as, hypertension, retinal arterial vasoconstriction, thromboembolism, haemorrhage, proteinuria, and intestinal perforation.[75-78] Despite local, ocular delivery in the treatment of neovascular AMD, little is known as to the potential systemic vasoactive effects these compounds. Additionally, within the eye, VEGF plays an important role in maintaining ocular health, where VEGF is involved in neuronal protection,[79] photoreceptor function,[80] and the formation and maintenance of healthy retinal vasculature.[81,82] The results of ongoing blockade of VEGF in animal models have demonstrated that inhibition of VEGF may lead to increased oxidative stress and an inflammatory response[83] and also significant closure of normal capillaries including the choriocapillaris.[77,84]

Additionally, it is not known whether measurement of retinal vascular calibre prior to treatment, may provide a marker of underlying disease severity in patients with neovascular AMD and may help to determine those eyes that are likely to fare less well with current anti-VEGF treatments and where alternative strategies may be useful.

TREATMENT REGIMEN

Although anti-VEGF agents have significantly improved the visual prognosis in patients with neovascular AMD, by inhibiting the exudative effects of VEGF on the choroidal vessels, there are theoretical concerns and logistic problems associated with the use of these drugs. Monthly dosing of anti-VEGF therapy is currently the

gold standard in the treatment of neovascular AMD. This followed the results of the pivotal ANCHOR and MARINA studies, which mandated the use of monthly injections of ranibizumab. Monthly dosing is inconvenient to patients and their carers, it creates logistic problems for physicians and is a considerable financial burden on the health care system. Secondly, each intravitreal injection carries with it a small but potentially devastating risk of endophthalmitis or severe inflammation within the eye.

As a result, of the desire to limit injection frequency, many clinicians tried varying the protocol in an attempt to reduce the treatment burden (reduced number of injections and follow up visits required), whilst achieving the same visual results as the trials mandating rigorous monthly injections.

The industry sponsored PIER and EXCITE studies investigated the 12-month efficacy of quarterly dosing of ranibizumab after three consecutive monthly injections. Both studies demonstrated that quarterly dosing regime was less effective than monthly treatment.

Other approaches being trialed were based, not upon fixed dosing schedules but around the individual patient responses guiding the treatment frequency, were more successful. Results from the small, PrONTO study, suggested that a flexible dosing regimen, with the OCT-measured central retinal thickness and VA being used as guiding criteria for re-treatment, might be as effective as monthly treatment.[85] Similar results were also seen in the SUSTAIN study,[37] where treatment outcome was similar to the monthly pivotal trials, although with a lower average number of re-treatments (mean of 5.6 injections over 12 months). A small loss in VA however, was seen after the initial gain from 3 monthly loading doses.

More recently clinicians have tried protocols based upon a “treat and extend” concept whereby, after three monthly loading dose injections, if the macula is “dry”, vision

stable and without signs of new haemorrhage, treatments continue to be given, although the follow-up interval is increased at each visit, up to a maximum of 12 weeks.[86] At any stage, if there are signs of CNV activity, an injection is given, with a subsequent reduction in the treatment interval. Where the macula is never cleared of fluid, monthly injections are continued, unless continuation is considered futile (fibrosis or atrophy).

To date, there are limited studies assessing visual outcomes in patients treated with this strategy. Oubraham et al.[87] showed a better BCVA outcome (+8.5 letters gain from baseline at 12 months) compared to similar patients treated with PRN injections.[88] Two further retrospective case series also reported promising outcomes with a treat and extend regimen of ranibizumab and bevacizumab in exudative AMD.[34,89] The significant visual improvements in these studies were achieved with fewer injections compared with monthly regimen.

The recently presented *LUcentis C*ompared to *A*vastin Study (LUCAS) trial also confirmed that both ranibizumab and bevacizumab were effective in improving vision by a mean of +8.2 and +8.0 letters respectively. This was achieved with a mean of 8.0-8.8 injections over 1 year (American Academy of Ophthalmology 2013 Annual Meeting).

With the variable outcomes of anti-VEGF in neovascular AMD incompletely understood, the main focus of this work is to further identify possible determinants that affect the response to anti-VEGF injections. Thus potential patient and treatment characteristics at baseline were assessed in a population of patients with neovascular AMD, who were actively undergoing anti-VEGF injections in a public hospital clinic. These patients were then prospectively followed in the clinics where

they underwent usual care so that “real life” determinants of visual acuity outcomes could be studied over a two-year period.

CHAPTER TWO

METHODS

STUDY POPULATION

All patients were recruited prospectively from the Medical Retina Clinics at the Royal Victorian Eye and Ear Hospital, Melbourne, Australia as part of an ongoing project looking at the predictors of outcome in patients with neovascular AMD, undergoing anti-VEGF treatment.

All patients with subfoveal/ juxtafoveal choroidal neovascularisation, secondary to AMD, who fitted the inclusion criteria, were recruited over a period between 2006 and 2010. Diagnosis of neovascular AMD was made on the basis of a dilated fundus examination, FFA and time domain OCT. Written informed consent was obtained in all cases and the Human Research Committee at the RVEEH approved the study. This research adhered to the tenets of the Declaration of Helsinki.

Inclusion criteria:

For inclusion, patients had to meet the following criteria: (1) age ≥50 years, (2) diagnosis of active subfoveal CNV secondary to AMD, which required the presence of leakage, as seen on FFA, and (3) have baseline VA of 25 to 73 ETDRS letter scores (Snellen equivalent of 20/320 to 20/40), measured with ETDRS charts at an initial distance of 4 meters by authorised VA examiners.

Exclusion criteria:

All patients with any previous anti-VEGF treatment, laser photocoagulation or verteporfin photodynamic therapy in the study eye, were excluded, as were those with CNV secondary to non-AMD conditions such as angioid streaks, severe myopia≥ -8 dioptres, central serous retinopathy or hereditary retinal disorders in either eye. Both eyes of patients if diagnosed with neovascular AMD, could be recruited.

Any occurrence of adverse events such as inflammation, endophthalmitis, raised intraocular pressure, retinal detachment and cataract were collected in all patients.

TREATMENTS

Treatment included 0.5mg ranibizumab (Lucentis; Novartis Pharma AG, Basel, Switzerland, and Genentech Inc, California, USA) or 1.25mg bevacizumab (Avastin; Novartis Pharma AG, Basel, Switzerland, and Genentech Inc, California, USA) using a "treat and extend" protocol in most cases. In Australia, ranibizumab is provided free of charge under the government pharmaceutical benefit scheme, for patients with active subfoveal CNV secondary to AMD. The approval process can delay the start of treatment, which may result in deterioration of vision.[90] To avoid this, bevacizumab was used for some patients as the first injection with subsequent ranibizumab treatments once approval was obtained. No patient was treated with bevacizumab alone.

IMAGING TECHNIQUES

OPTICAL COHERENCE TOMOGRAPHY

This is an optical signal acquisition and processing method. It captures micrometre-resolution, three-dimensional images from within optical scattering media (e.g., biological tissue). OCT is an interferometric technique, typically employing near-infrared light. The use of relatively long wavelength light allows it to penetrate into the scattering medium, thus obtaining sub-surface images of translucent or opaque materials at a resolution equivalent to a low-power microscope. It is effectively

‘optical ultrasound’, imaging reflections from within tissue to provide cross-sectional images (Figure 10).

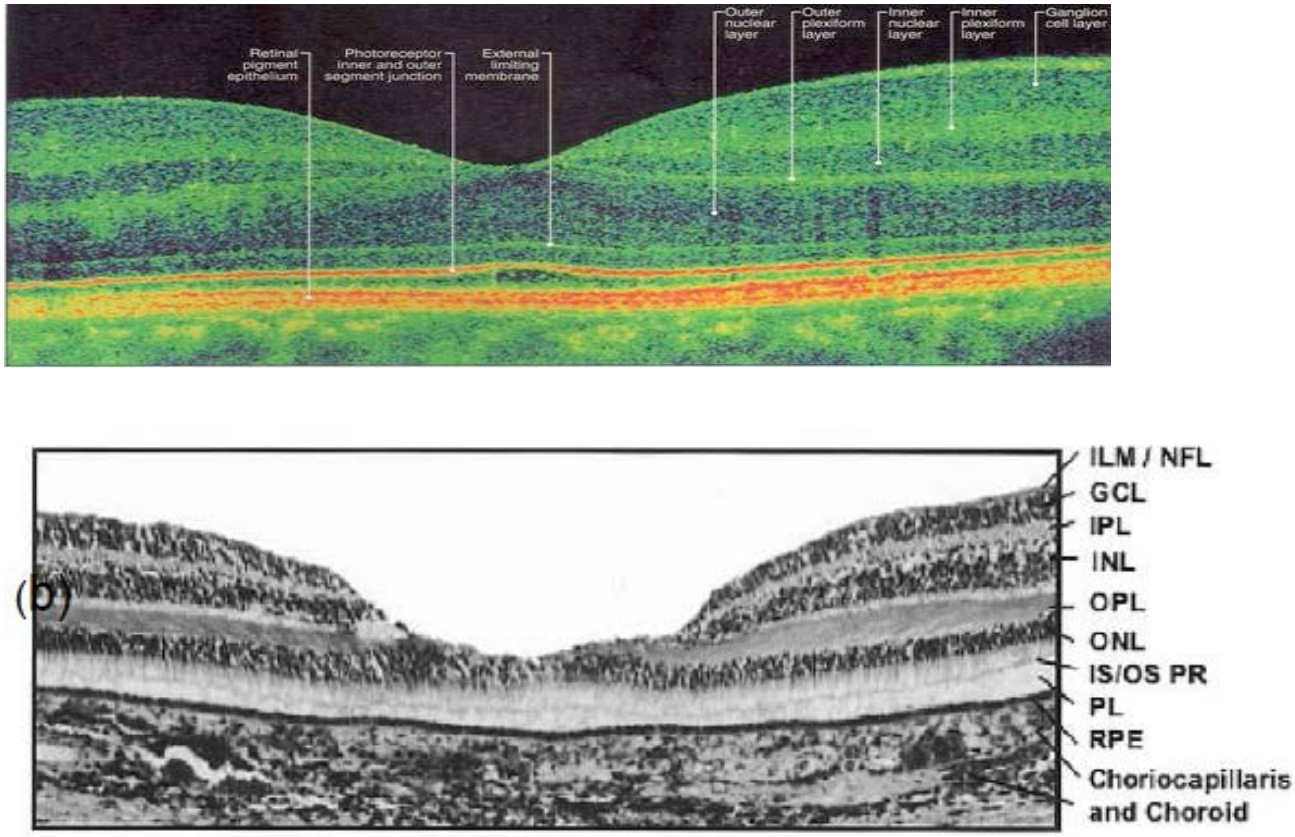


Figure 10. Spectral domain optical coherence tomography and histological section of a healthy macula

There are two main categories of OCT instrumentation: Time-Domain OCT and Spectral-Domain OCT. Swept source OCT is another modality, although is largely confined to research at present. Most early research was based on Time Domain OCT, although due to the significant advantages in sensitivity and imaging speed, Spectral-Domain OCT is currently the standard of care in OCT imaging. Since patient

recruitment began in 2006/7, most of the publications included in this thesis utilised Time domain OCT in the determination of central macular thickness and qualitative assessment of the macula (OCT 3 Stratus, Zeiss-Humphrey INC., San Leandro, CA). In all cases an experienced technician, using the radial lines protocol with an additional 6.0mm cross hair scan to allow a qualitative assessment of the macular anatomy. In the course of follow up, a spectral domain OCT was introduced into our unit and thus later patients did have imaging with this instrument, Cirrus (Zeiss-Humphrey INC., San Leandro, CA).

A masked observer made qualitative assessment of the OCT scans and were scored for the presence (on any of the radial line scans or a 6mm cross hair) of intraretinal fluid (cysts or oedema), subretinal fluid, pigment epithelial detachments and the presence of a significant band of reflectance at the retinal pigment epithelial level.

FUNDUS FLUORESCEIN ANGIOGRAPHY

The diagnosis of choroidal neovascularisation, secondary to neovascular AMD was made with fundus fluorescein angiography, using a standard injection of 5mls of 10% intravenous Sodium Fluorescein ($C_{20}H_{10}O_5Na_2$). A series of digital photographs of the fundi were taken over a period of 5 minutes, to determine the presence of choroidal vascular leak as leakage characteristics and site of leakage in relation to the fovea. All photographs were taken using a Topcon fundus camera, TRC50 EX, Tokyo, Japan.

RETINAL VASCULAR ASSESSMENT

Patients had an optic disc centred photograph taken of each eye at baseline and then at three monthly intervals up to 12 months. Retinal vascular calibre was measured using a computer-based program (IVAN, University of Wisconsin, Madison). For each photograph (Figure 11), all arterioles and venules coursing through an area one-half to one-disc diameter from the optic disc margin were measured and vessel calibres of the biggest six were summarized as the central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE). These equivalents are projected calibres for the central retinal artery and vein.[91,92]

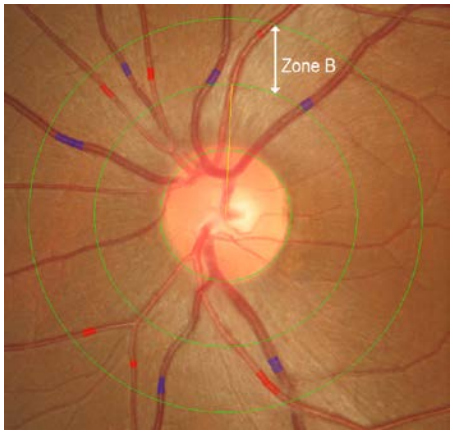


Figure 11. Disc centred photograph of the left optic nerve head, highlighting the "Big 6" arterioles and venules in Zone B, used to calculate the Central retinal artery equivalent (CRAE) and Central retinal vein equivalent (CRVE).

GENETIC ANALYSIS

Complement genes, HTRA1, LOC387715/ ARMS2

A peripheral blood sample was obtained from each patient and a total of 17 SNPs in known AMD-associated genes, including SNPs rs800292, rs3766404, rs1061170, rs2274700, and rs393955 in the CFH gene, SNP rs11200638 in HTRA1, SNPs rs10922153, rs16840639, rs6667243, and rs1853883 in CFHR1-5 genes, SNPs

rs3793917 and rs10490924 (A69S) in LOC387715/ARMS2, SNPs rs2230199 and rs1047286 in C3, SNP rs547154 in C2, SNP rs641153 in CFB and SNP rs6003 in F13B, were genotyped.

Genotyping was performed on the MassArray platform (SEQUENOM, San Diego, CA) at the Murdoch Children's Research Institute, Melbourne, Victoria, Australia. Briefly, 10 ng of genomic DNA was amplified using polymerase chain reaction. A MassEXTEND reaction was initiated by adding a Hotstart DNA polymerase (Bioline, Taunton, MA) and a common primer that allowed 1 base pair primer extension of either allele at the polymorphic site using the designed primers. Deoxynucleotides incorporated at the polymorphic site were terminated with the incorporation of a di-deoxynucleotide, and excess nucleotides were removed through the use of shrimp alkaline phosphatase. Using this approach, 2 allele-specific products of different mass were generated. Samples were conditioned using SpectroCLEAN (Sequenom, San Diego, CA) resin to remove excess salt that might interfere with MALDI-TOF analysis. Each sample was transferred from a 384-well microtiter plate and spotted onto the pad of a 384 SpectroCHIP II microarray. The SpectroCHIP II was placed into the MALDI-TOF and genotypes were simultaneously called in real time with SpectroTYPERTM software.

Apolipoprotein E (APOE)

The single nucleotide polymorphisms (SNP) rs429358 and rs7412 of the *ApoE* gene were genotyped. In brief, the Polymerase chain reaction (PCR) amplification was undertaken with the primers AF(5'GCCTCCCCACTGTGCGA3'), AR(5'GGCCGAGCATGGCCTG3') for rs429358 and BF(5'ACCGAGGAGCTGCGGG3'), BR(5'CTCGCGGATGGCGCTGA3') for rs7412. Digestion of each PCR product was undertaken with the restriction enzymes *Afl*III for rs429358 and *Hae*II for rs7412. Digested products were size separated on agarose gels. The combination of amino acids at these two SNPs allowed the common allelic variants of either ε2, ε3, or ε4 of the *ApoE* gene to be determined. Dideoxynucleotide sequencing was undertaken on a representative sample to confirm the results of each restriction digestion profile.

VEGF

Seven SNPs—rs3024994, rs3025000, rs3025042, rs3025047, rs3025035, rs3025030, and rs3025010—in the VEGFA gene were identified through the selection of tSNPs via the use of the International HapMAP Project (http://www.hapmap.org/; accessed July 20, 2010). The tSNPs were selected using the pairwise algorithm and a CEU (Utah residents with ancestry from northern and western Europe) population. A strong linkage disequilibrium tagging criteria of r2_0.8 was used and a minor allele frequency of 0.01 was chosen.

CHAPTER THREE

RESULTS

1. PATIENT CHARACTERISTICS

Delay to treatment and visual outcomes in patients treated with anti-vascular endothelial growth factor for age-related macular degeneration.

Lim JH, **Wickremasinghe SS**, Xie J, Chauhan DS, Baird PN, Robman LD, Hageman G, Guymer RH. Am J Ophthalmol. 2012 Apr;153(4):678-86

PURPOSE: To investigate the potential influences that affect visual acuity (VA) outcome in a clinic-based cohort of age-related macular degeneration (AMD) patients undergoing anti-vascular endothelial growth factor (anti-VEGF) treatment for choroidal neovascularization.
DESIGN: Prospective interventional case series.
METHODS: Patients with subfoveal choroidal neovascularization (CNV) secondary to AMD were prospectively recruited. A detailed questionnaire was given to patients at time of enrollment, to collect information relating to demographics, history of visual symptoms, visual acuity (VA), and treatment scheduling. Delay from symptoms to treatment ("Treatment delay") was measured in terms of weeks and analyzed in tertiles. Information pertaining to treatment outcomes was collected over a 6-month period.
RESULTS: One hundred eighty-five eyes of 185 patients were recruited into the study. Longer delay from first symptoms suggestive of CNV to first injection was a significant predictor (P=.015) of poorer treatment outcome, when controlling for age, sex, and baseline VA. Patients with a delay in treatment of 21 weeks or more compared to a delay of 7 weeks or less had an odds ratio of 2.62 (1.20, 5.68) for worsening vision after treatment.
CONCLUSIONS: Patients experiencing a longer delay between their first symptoms of CNV and their first anti-VEGF treatment have a significantly lower chance of improving vision at 6 months following anti-VEGF therapy. It is critical that this information reach those at potential vision loss from AMD, in order that prompt treatment may be instituted, to maximize the benefits of anti-VEGF treatment.

DELAY IN TREATMENT INITIATION FROM FIRST SYMPTOMS

It has been identified that larger areas of CNV adversely affect visual outcome following anti-VEGF injections. This may be as a result of chronicity, since larger lesions are more likely to have been present for longer periods, thus potentially leading to chronic and possibly irreversible damage to the outer retina and RPE.

Accurately determining the time interval between initial, symptoms suggestive of CNV (metamorphopsia, central blur, central scotoma) and first anti-VEGF treatment is difficult. Often patients are unsure of the timing of initial symptoms, especially where visual acuity is good in the fellow eye. However it is this time, from first onset of symptoms to the eventual treatment that is likely to be quite variable and in some cases prolonged.

In our cohort, we studied 185 eyes of 185 patients who had detailed questioning regarding the timing of the onset of symptoms of neovascular AMD, such as when the patient first described metamorphopsia, new onset of central blur, or central scotoma. This information was collected by a retinal clinician at the time of diagnosis, to try to minimize incorrect interpretation of patient symptoms. Treatment delay from the time of the "first symptom" was measured in terms of weeks and the time from angiographic diagnosis to first treatment was also noted. All patients were treated using the modified protocol as defined by the SUSTAIN trial (previously described).

Individuals were recruited for subgroup analysis based on the following characteristics: (i) age [≤74, 75 – 84, ≥85 years]; (ii) gender [male or female]; (iii) smoking status [non-smoker, past/current smoker]; (iv) treatment delay between first suggestive CNV symptoms and first treatment; (v) treatment delay between angiographically confirmed CNV and first treatment; (vi) CNV lesion types [predominantly classic, non-predominantly classic]; (vii) lesion size [≤2 disc areas

[DA], >2 DA]; (viii) injection number [≤3, >3]; and (ix) baseline VA. All of these treatment subgroups were selected before study commencement.

Results

We found that increasing delays were associated with adverse visual outcome despite anti-VEGF treatment. Patients with a delay in treatment of greater than 21 weeks were 2.6 times less likely to gain vision than those with a 7-week or shorter delay, after adjusting for age, gender and baseline vision. Interestingly, the chance of a 10 letter loss in vision was not influenced by the delay with 11% in eyes with <7 weeks delay compared to 13% in those with a delay of >21 weeks. This suggests that reducing delays are imperative in order to maximise the gain in acuity that may be achieved with anti-VEGF treatment, but that other factors were involved in determining those that lost vision.

Additionally, our study also confirmed previous findings that, increasing age and better vision were more likely to lead to loss of more letters, following anti-VEGF treatment. We did not however find any significant associations between larger CNV lesions and worse visual outcomes. Similarly lesions with a classic component appeared to fare just as well as occult lesions, where the vessels are thought to remain under the RPE.

Thus we did not find fluorescein angiography characteristics a useful differentiating factor in determining treatment outcome with anti-VEGF injections.

Discussion

It would appear that the mere presence of choroidal neovascularisation leading to fluid accumulation in the intra and/or subretinal space leads to photoreceptor/ RPE damage. Increasing duration of symptoms may be a good correlate of damage to the outer retina and retinal pigment epithelium. Thus, increasing delay may predict a reduced likelihood to improve visual acuity with treatment with greater likelihood of permanent damage to the photoreceptor/ retinal pigment epithelium complex. With greater disruption of the photoreceptors and neural connections within the retina, anti-VEGF may be less effective in improving vision due to irreversible damage to the outer retina, even if there is resolution of fluid from the macula. Prompt treatment may reduce the disruption of macular anatomy and may improve functional visual outcomes with anti-VEGF treatment.

We concluded that education of optometrists and primary care physicians as well as patients was important to ensure prompt referral of patients with distortion and reduced vision, in order that anti-VEGF treatment results could be optimised.

2. IMAGING CHARACTERISTICS

OPTICAL COHERENCE TOMOGRAPHY

Predictors of AMD treatment response.

Wickremasinghe SS, Sandhu SS, Busija L, Lim J, Chauhan DS, Guymer RH. Ophthalmology. 2012 Nov;119(11):2413-2414.

Letter to Editor

Although central macular thickness has not been found to correlate well with visual acuity,[93] the location of fluid on OCT may better predict visual acuity outcome as it may correlate better with the likely underlying damage to the retina / retinal pigment epithelium (RPE).[94] It is well known that SRF in conditions such as CSCR may be present for extended periods of time without leading excessive vision loss or permanent reduction in vision on resolution of the fluid. IRF on the other hand in diabetic macular oedema or retinal vein occlusion may lead to permanent reduction in vision, despite resolution of fluid. This is often associated with the appearance of pigment changes in the macula.

The vitality of the RPE and photoreceptor cell layer and its association with anti-VEGF treatment outcomes in neovascular AMD needs to be explored. In a large, pooled analysis of participants undergoing anti-VEGF treatment for neovascular AMD, vision loss after two years of treatment was associated with signs of RPE dysfunction such as pigmentary abnormalities and atrophic scars in the absence of leakage on fluorescein angiogram.[42] In two other smaller studies, RPE abnormalities, as seen on fundus autofluorescence were the only factors independently associated

with poorer treatment outcomes.[45,95] Pigment epithelial detachment (PED) at baseline has been reported to lead to a higher risk of secondary loss of vision at follow-up after three, monthly injections of RBZ.[96]

In order to further evaluate the potential role of OCT in predicting outcomes in the treatment of noeovascular AMD, we studied qualitative features using time domain OCT in 214 eyes of 144 patients. All patients received three, monthly injections of ranibizumab and/or bevacizumab, with subsequent retreatment decisions at monthly visits based on a modification of the SUSTAIN study, where retreatment was performed if there was either persistence or recurrence of intraretinal fluid or subretinal fluid on OCT, loss of ≥1 lines of vision, or the presence of new macular haemorrhage on clinical examination.

Results

1. Location of fluid

We found that in our patients, the presence of intraretinal cystic oedema on OCT, at baseline, was associated with worse visual outcome at 12 months, compared to where only subretinal fluid (SRF) was present. Similarly, the failure to clear intraretinal fluid (IRF) from the macula, following three, monthly anti-VEGF injections, was predictive of a six fold greater chance of vision loss at 12 months, compared to where IRF was cleared after initial treatment.[94]

2. Integrity of the Retinal pigment epithelium

In 29 of the 214 eyes that we recruited, significant hyper-reflectance at the level of the RPE (Figure 12) was visible. We found that this was strongly associated with worse visual outcomes regardless of whether there was associated SRF or IRF. Most of the eyes with this feature in our cohort had significant subretinal haemorrhage at baseline and went on to develop subretinal fibrosis and/ or atrophy through the course of treatment. When significant hyper-reflectance was visible and eyes were otherwise free of fluid, half the eyes were noted to have atrophy of the RPE and outer retina. When associated with fluid, most eyes had significant subretinal fibrotic changes noted clinically. The hyper-reflective band may therefore represent loss of photoreceptors with neural disorganization preventing visual improvement regardless of the presence or absence of fluid. Thus this feature, when observed predicted a worse outcome for vision, even when IRF or SRF was absent.

We found no association of the presence of a PED and visual outcomes, although there was a possible trend for worse outcome in eyes where a PED was present at baseline.

Discussion

The presence of IRF and poor visual outcomes may relate to disruption of the retinal elements as opposed to where only SRF is present, where there maybe relatively intact retinal architecture. The clearance of IRF may lead to significant visual improvement, suggesting that at least in some cases the structural changes are reversible, however failure to resolve fluid from the retina after three injections may be a marker of more irreversible structural damage to the photoreceptor/ retinal pigment epithelium complex and thus may explain the strong association with adverse visual outcome at 12 months. The presence of SRF, although not desirable

may be better tolerated and may only lead to loss of vision, when present for extended periods or when there is an increase in fluid height.

The presence of significant hyper reflectivity may also give a prognostic indication of how likely anti-VEGF treatment is to improve vision and thus provide help to clinicians in counselling the patient and determining how aggressive anti-VEGF treatment should be. The hyper-reflective band may represent loss of photoreceptors with neural disorganization preventing visual improvement regardless of the presence or absence of fluid.

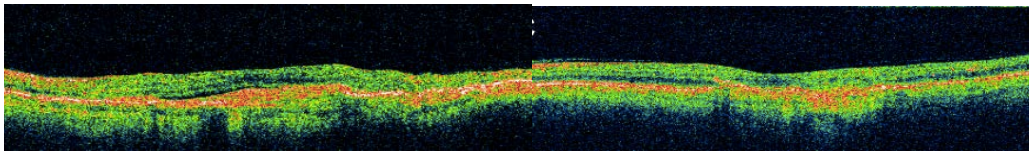


Figure 12. OCT appearance showing hyper reflective band at the level of the retinal pigment epithelium (A) with associated subretinal fluid, (B) without subretinal or intraretinal fluid.

3. GENETIC FACTORS

COMPLEMENT FACTOR H GENE

Genetic influences on the outcome of anti-vascular endothelial growth factor treatment in neovascular age-related macular degeneration.

Abedi F, **Wickremasinghe S**, Richardson AJ, Islam AF, Guymer RH, Baird PN.

Ophthalmology. 2013 Aug;120(8):1641-8.

PURPOSE: To determine the association of genetic variants in known age-related macular degeneration (AMD) risk-associated genes with outcome of anti-vascular endothelial growth factor (VEGF) treatment in neovascular AMD.
DESIGN: Prospective cohort study.
PARTICIPANTS: We enrolled 224 consecutive patients with neovascular AMD at the Royal Victorian Eye and Ear Hospital, Australia.
METHODS: Patients were treated with 3 initial monthly ranibizumab or bevacizumab injections followed by 9 months of "as required" injections based on clinician's decision at each follow-up visit according to retreatment criteria. Seventeen single nucleotide polymorphisms (SNPs) in known AMD risk-associated genes including CFH (rs800292, rs3766404, rs1061170, rs2274700 and rs393955), HTRA1 (rs11200638), CFHR1-5 (rs10922153, rs16840639, rs6667243, and rs1853883), LOC387715/ARMS2 (rs3793917 and rs10490924), C3 (rs2230199 and rs1047286), C2 (rs547154), CFB (rs641153) and F13B (rs6003) were examined. Multivariate analysis was used to determine the role of each SNP in treatment outcome.
MAIN OUTCOME MEASURES: The influence of selected SNPs on mean change in visual acuity (VA) at 12 months.
RESULTS: Mean baseline VA was 51 ± 16.8 Early Treatment Diabetic Retinopathy Study letters. Overall, the mean change in VA from baseline was +3.2 ± 14.9 letters at 12 months. The AA (homozygote risk) genotype at rs11200638 - HTRA1 promoter SNP (P = 0.001) and GG (homozygote risk) genotype at rs10490924 (A69S) in LOC387715/ARMS2 (P = 0.002) were each significantly associated with poorer VA outcome at 12 months after multiple correction. Mean ± standard deviation change in VA from baseline in patients with AA genotype at rs11200638 was -2.9 ± 15.2 letters after 12 months compared with +5.1 ± 14.1 letters in patients with AG or GG genotypes at this SNP. Patients with either of these genotypes were also significantly more likely to lose >15 letters after 12 months. SNPs rs11200638 and rs10490924 were in high linkage disequilibrium (r(2) = 0.92). None of the other examined SNPs was associated with outcome.
CONCLUSIONS: The HTRA1 promoter SNP (rs11200638) and A69S at LOC387715/ARMS2 were associated with a poorer visual outcome for ranibizumab or bevacizumab treatment in neovascular AMD, suggesting strong pharmacogenetic associations with anti-VEGF treatment. This finding could aid in applying more individualized treatment regimens based on patients' genotype to achieve optimal treatment response in AMD.

Several variants of the *CFH* gene have been identified in people with AMD. The Tyr402His (Y402H) has been associated with an increased risk of the condition. People who carry one copy of this polymorphism have a 2.5-fold increased risk of developing age-related macular degeneration compared to people who do not have the polymorphism, and people who carry two copies of the polymorphism have a six-fold increased risk.

The effect on CFH gene polymorphisms on the visual outcome in neovascular AMD is not established, with some studies showing an association, whilst in others no apparent association has been noted.[48,49,51,60]

AGE-RELATED MACULOPATHY SUSCEPTIBILITY 2 (ARMS2)/ HIGH-TEMPERATURE REQUIREMENT A-1 GENES (HTRA1)

The protein products of the *LOC387715/ARMS2* gene *and the HTRA1* gene are thought to be involved in extracellular matrix function.

Similar to the CFH polymorphisms, variants in the *LOC387715/ARMS2* gene have also yielded variable results with respect to treatment outcome in neovascular AMD treated with anti-VEGF injections.[49,50,54,97]

Methods

To further investigate potential pharmacogenetic influences on anti-VEGF treatment for neovascular AMD, we investigated the influence of single nucleotide polymorphisms (SNPs) in known AMD risk-associated genes including *CFH* (rs800292, rs3766404, rs1061170, rs2274700 and rs393955), *HTRA1* (rs11200638), *CFHR*1-5 (rs10922153, rs16840639, rs6667243, and rs1853883), *LOC387715/ARMS2* (rs3793917 and rs10490924), *C3* (rs2230199 and rs1047286),

C2 (rs547154), *CFB* (rs641153) and *F13B* (rs6003) in determining changes in vision after 12 months of anti-VEGF treatment.

To assess the contribution of genetic variation within the complement associated genes as well as the *LOC387715/ARMS2* gene, we recruited 224 consecutive patients with neovascular AMD, treated with anti-VEGF agents in protocol modified from the SUSTAIN trial (following three, monthly loading dose injections). In these patients, we assessed visual outcome of patients at 12 months, by characterizing them by their genotype.

Results

Overall in our study, the mean change in VA from baseline was +3.2± 14.9 letters at 12 months. Counter to some other studies, we found no significant associations with visual outcome following anti-VEGF treatment and any of the complement pathway genes. Thus we suggest that these genes may be more associated with the development of AMD, rather than influencing the treatment outcome of the disease.

Conversely in our patients, we found that the AA (homozygote risk) genotype at rs11200638 - *HTRA1* promoter SNP (P = 0.001) and GG (homozygote risk) genotype at rs10490924 (A69S) in *LOC387715/ARMS2* (P = 0.002) were each significantly associated with poorer visual outcome at 12 months. Patients with either of these genotypes were also significantly more likely to lose >15 letters.

With respect to the rs11200638 SNP, the presence of the AA genotype was associated with a vision loss of -2.9 ± 15.2 letters from baseline to 12 months compared with a gain of +5.1 ± 14.1 letters in patients with AG or GG genotypes at this SNP.

Discussion

Our results suggest a strong pharmacogenetic association with anti-VEGF treatment.

It has been reported that enhanced transcription of HTRA1 occurs in patients with the AA genotype at SNP rs11200638. The HTRA1 protein, expressed in human retina, is a heat shock protein in the serine protease family and seems to regulate degradation of the extracellular matrix proteoglycans.[14,98] As a result, overexpression of HTRA1 may affect the integrity of Bruch' s membrane and potentiate the invasion of choroidal blood vessels across the extracellular matrix.[14] In addition, HTRA1 inhibits transforming growth factor-β, which regulates angiogenesis.[99] Loss of integrity of Bruch' s membrane and/or angiogenesis subsequent to HTRA1 overexpression, driven by the AA genotype at the HTRA1 promoter SNP may contribute to the underlying biological mechanisms for a poor response to intravitreal anti-VEGF treatment.

With respect to the possible biological mechanism for the effect of SNP rs10490924 (A69S) in the treatment outcome, localization of ARMS2 protein to the outer membrane of mitochondria suggests that the effect could be through oxidative damage of photoreceptors and retinal pigment epithelium.[100]

These findings could aid in applying more individualized treatment regimens based on patients' genotype to achieve optimal treatment responses in neovascular AMD treatment.

Variants in the VEGFA gene and treatment outcome after anti-VEGF treatment for neovascular age-related macular degeneration.

Abedi F, Wickremasinghe S, Richardson AJ, Makalic E, Schmidt DF, Sandhu SS, Baird PN, Guymer RH.

Ophthalmology. 2013;120(1):115-21.

PURPOSE: To determine the association of genetic variants of the VEGFA gene with outcome of anti-vascular endothelial growth factor (VEGF) treatment in neovascular age-related macular degeneration (AMD).
DESIGN: A prospective cohort study.
PARTICIPANTS: We included 201 consecutive patients receiving anti-VEGF injections for neovascular AMD.
METHODS: Patients were followed over 12 months. They were treated with 3 initial monthly ranibizumab or bevacizumab injections. Thereafter, the decision to retreat was made by clinicians at each follow-up visit on the basis of retreatment criteria. Seven tagged single nucleotide polymorphisms (tSNPs) in the VEGFA gene were selected and examined. Multivariate data analysis was used to determine the role of each tSNP in treatment outcome.
MAIN OUTCOME MEASURES: The influence of selected VEGFA tSNPs on visual acuity (VA) outcome at 6 months.
RESULTS: Mean baseline VA was 51±17 Early Treatment Diabetic Retinopathy Study (ETDRS) letter scores. Overall, the mean change in VA from baseline was +6.5±12, +4.4±13.4, and +2.3±14.6 letters at 3, 6, and 12 months, respectively. The tSNP rs3025000 was the only SNP significantly associated ($P<1 \times 10(-4)$) with visual outcome at 6 months with multiple correction. The presence of the T allele (TC or TT genotypes) at this tSNP predicted a better outcome of +7 letters at 6 months compared with the CC genotype. In a subgroup analysis, presence of the T allele predicted a significantly higher chance of the patients belonging to the responder group (gain of ≥5 letters from baseline) after 3, 6, and 12 months treatment (odds ratio, 2.7, 3.5, and 2.4; 95% confidence interval, 1.46-5.07, 1.82-6.71, and 1.27-4.57, respectively) than any other outcome group.
CONCLUSIONS: Pharmacogenetic association with anti-VEGF treatments may influence the visual outcomes in neovascular AMD. In patients with the T allele in tSNP rs3025000, there was a significantly better visual outcome at 6 months and a greater chance of the patients belonging to the responder group with anti-VEGF treatment at 3, 6, and 12 months. The VA outcomes of patients harboring the T allele at SNP rs3025000 were comparable with those of the pivotal clinical trials but with fewer injections, making the treatment perhaps more cost effective in certain subgroups of patients.

Since VEGF is a key factor in vasculogenesis in neovascular AMD, variants in the *VEGF* gene may influence the levels of VEGF in the eye, or binding affinity with drugs and thus, may influence the results of anti-VEGF blockade and as a result treatment outcome following injections.

Methods

In our cohort, we studied the influence of 7 SNPs within the *VEGF* gene in 201 patients with neovascular AMD, to assess if there was any association with any of these SNPs and visual outcome at 12 months. Similar to our other studies, all patients were treated with anti-VEGF injections using a modified SUSTAIN trial protocol, to determine the need for treatment after 3, monthly injections.

Results

We found that variants of the SNP at position rs3025000 of the *VEGF* gene, were associated with vision outcomes at 6 months. The presence of the T allele (TC or TT genotypes) at this SNP predicted a better outcome of +7 letters at 6 months compared with the CC genotype. In a subgroup analysis, presence of the T allele predicted a significantly higher chance of the patients belonging to the responder group (gain of ≥5 letters from baseline) after 3 months (OR, 2.7; 95% confidence interval [CI], 1.46 –5.07; $p<0.001$), 6 months (OR, 3.5; 95% CI, 1.82– 6.71; $p<0.0001$)) and 12 months treatment (OR, 2.4; 95% CI, 1.27– 4.57; $p=0.006$). Thus we found that variants of this SNP influence the outcome of anti-VEGF treatment. Patients with a T allele achieved similar visual outcomes compared to the pivotal clinical trials, but with fewer injections.

Discussion

The biological mechanism for this finding is not understood but one possible mechanism may be related to underlying hypoxia. A significant association has previously been reported between the rs3025000 SNP variants and acute mountain sickness (AMS). Patients with AMS had significantly lower peripheral oxygen saturation levels at high altitude compared with those with those without AMS.[101] The mechanism of development of AMS is not fully understood, however, hypoxemia secondary to hypoxia and subsequent vascular changes in the body are the main pathophysiological changes seen in AMS.[102] Other studies have shown that patients with AMS have significantly higher free plasma VEGF-A levels at high altitude compared with healthy individuals, suggesting a role for increased free plasma VEGF-A levels in the pathophysiology of AMS.[103] It is also known that hypoxia is a major inducer of *VEGF* gene transcription.[26] The association of the genetic variant rs3025000 with both AMS and the response to anti-VEGF treatment in neovascular AMD suggests that rs3025000 in the presence of hypoxia may increase VEGF-A levels or function and therefore affect the efficacy of VEGF antagonism, in the treatment of neovascular AMD.

Variants in the APOE gene are associated with improved outcome after anti-VEGF treatment for neovascular AMD.

Wickremasinghe SS, Xie J, Lim J, Chauhan DS, Robman L, Richardson AJ, Hageman G, Baird PN, Guymer R. Invest Ophthalmol Vis Sci. 2011; 52(7):4072-9.

PURPOSE: Anti-vascular endothelial growth factor (anti-VEGF) drugs have dramatically improved the treatment of neovascular AMD. In pivotal studies, almost 90% of patients maintain vision, with approximately 30% showing significant improvement. Despite these successes, 10% to 15% of patients continue to lose vision, even with treatment. It has been reported that variants in some AMD-associated genes influence treatment outcome. This study showed an association of treatment outcome with variants in the apolipoprotein E (APOE) gene.

METHODS: One hundred ninety-two patients receiving anti-VEGF treatment for subfoveal choroidal neovascularization secondary to AMD were enrolled. Information on demographics, lesion characteristics, delay until treatment, visual acuity (VA), and number of treatments was collected, and variants of APOE were assessed in all patients at baseline. Best corrected logarithm of the minimum angle of resolution (logMAR) VA was recorded in all patients.

RESULTS: The presence of the APOE ε4 allele was associated with improved treatment outcome at 3 (P = 0.02) and 12 (P = 0.06) months, compared with the presence of the ε2 allele, after adjustment for baseline acuity, treatment delay after first symptoms, age, and sex. Patients with an APOE ε4 allele had an odds ratio (OR) of 4.04 (95% confidence interval [CI], 1.11-14.70) for a 2-line gain in vision from baseline at 3 months (P = 0.03) and an OR of 2.54 (95% CI, 0.61-10.52; P = 0.20) at 12 months after treatment, based on multivariate analysis.

CONCLUSIONS: In patients with neovascular AMD, the presence of the APOE ε4 allele conferred significantly better visual outcomes after anti-VEGF treatment than did the ε2 allele. These findings suggest a possible role for a personalized approach to treatment with anti-VEGF.

The *ApoE* gene is mapped to chromosome 19 and is polymorphic with three major isoforms: *ApoE2* (cys130, cys176), *ApoE3* (cys130, arg176), and *ApoE4* (arg130, arg176). E2 (rs7412) is found in approximately 7 percent of the population.[104] E2 is associated with the genetic disorder hyperlipoproteinemia type III and with both increased and decreased risk for atherosclerosis. Individuals with an E2/E2 combination may clear dietary fat slowly and be at greater risk for early vascular disease and type III hyperlipoproteinemia, where 94.4% of such patients are E2/E2.

E3 (rs429358) is found in approximately 79% of the population. It is considered the "neutral" Apo E genotype, whilst E4 is found in approximately 14% percent of the population.[104] E4 has been implicated in atherosclerosis and Alzheimer's disease, impaired cognitive function and reduced neurite outgrowth.[105]

With respect to AMD risk, several epidemiologic studies in different populations of AMD have shown associations between the ApoE polymorphisms and risk of developing AMD.[16-19] Seddon et al found that the Apo E4 allele frequency in patients with exudative AMD was lower than in subjects without the disease.[106]

Methods

In order to assess the influence of APOE variants on the outcome of anti-VEGF treatment for neovascular AMD, we studied 172 patients receiving anti-VEGF treatment for subfoveal choroidal neovascularization secondary to AMD. All patients were treated with anti-VEGF injections using a modified SUSTAIN trial protocol.

Results

In our cohort the putative AMD-risk E2 allele for the *APOE* gene was identified in 17.4% of all patients, whereas the protective E4 allele was present in 16.1 %. At three months, the presence of at least one E2 allele (E2 /E2 or E2/E3) was

associated with a two-line improvement in visual acuity in only 16.7% of carriers, whilst 45.5% of E4 (E 3/E4 or E4/E4) carriers showed this improvement. In patients with only the E3 allele (E3/E3), 27.5 % improved vision by two or more lines, p=0.06.

Looking specifically at the presence of the E2 allele compared with the presence of the E4 allele, there was a significant difference in outcome. The presence of the E4 allele was associated with a greater likelihood of vision improvement (p=0.02) at three months. A similar, although not significant trend was noted at six months and 12 months, with a greater percentage of patients with the E4 allele compared to the E2 allele showing vision improvement (33.3% vs. 12.3%, p=0.07 and 37.5% vs. 15.4%, p=0.06) at six and 12 months respectively.

In a multiple regression analysis, after adjusting for age and baseline visual acuity, we found that patients with an E4 allele had a four times greater chance of a 2-line gain in vision from baseline at 3 months.

Discussion

The mechanism underlying this differential response may relate to conformation changes in the structure of the APOE protein as coded for by variants of the gene. The protein coded for by E4 may allow better elimination of lipids through Bruch's membrane compared to E2 and E3. Since, unlike E2 and E3, E4 lacks cysteine residues at position 112 and 158, preventing the formation of large dimers and thereby allowing for easier clearance of lipids through Bruch's membrane.

Conversely, the APO E2 allele may also have potential harmful effects, Lee et al[107] demonstrated in their mouse model for neovascular AMD, that eyes from APO E2 mice, regardless of diet, had significant lipid accumulation within RPE, whereas control mice did not. Additionally, there was over-expression of vascular endothelial growth factor and basic fibroblast growth factor with the RPE of APO E2 eyes

compared with controls. Pigment epithelial derived factor (PEDF) expression was slightly decreased. These expression patterns of VEGF, bFGF, and PEDF suggest angiogenesis may be induced by APO E2 by altering angiogenic cytokines.

4. RETINAL VASCULATURE

Retinal venular caliber predicts visual outcome after intravitreal ranibizumab injection treatments for neovascular AMD.

Wickremasinghe SS, Busija L, Guymer RH, Wong TY, Qureshi S. Invest Ophthalmol Vis Sci. 2012;53(1):37-41.

PURPOSE: To examine whether baseline retinal vascular caliber predicts visual response to intravitreal ranibizumab injections in patients with neovascular age-related macular degeneration (AMD).

METHODS:
In this prospective cohort study, patients with neovascular AMD received three monthly intravitreal injections of ranibizumab, followed by as needed dosing up to 1 year. Retinal vascular caliber was measured from digital fundus photographs at baseline and summarized as central retinal artery equivalent (CRAE) and venular equivalent (CRVE), representing average caliber of arterioles and venules, respectively. Visual outcome at 12 months was assessed and the relation to baseline retinal vascular caliber was determined.

RESULTS:
A total of 88 eyes were analyzed at baseline. After accounting for age, sex, size of choroidal neovascularization, and number of injections, patients who deteriorated in visual acuity at 12 months had significantly larger baseline CRVE, 243.10 μm (95% confidence interval [CI], 227.01-259.19), compared with those who were stable, 214.30 μm (95% CI, 205.79-222.81) and those who improved, 215.26 μm (95% CI, 204.69-225.84; P = 0.007). Baseline CRAE did not differ significantly from eyes whose vision deteriorated, 150.12 μm (95% CI, 140.67-159.57), compared with those remaining stable, 143.64 μm (95% CI, 138.64-148.63), or gaining vision 142.92 μm (95% CI, 136.71-149.13; P = 0.69).

CONCLUSIONS:
In eyes with neovascular AMD treated with intravitreal ranibizumab, larger baseline retinal venular caliber was significantly associated with a poorer response to treatment, possibly reflecting increased disease severity.

Another possible cause for the differential response to anti-VEGF treatment in patients with neovascular AMD, may relate to the degree of underlying disease severity at baseline. Eyes with more severe disease may respond less well to treatment compared to eyes with less disruption.

Retinal vascular calibre is an indirect indicator of ocular blood flow that can be measured from fundus photographs. Changes in retinal vascular calibre have been linked with systemic as well as ocular diseases, and may reflect changes in retinal blood flow, inflammation, ischaemia and endothelial function. Although neovascular AMD is a consequence of the development of choroidal neovascularization. The underlying aetiology may relate to underlying ischaemia and inflammation in the choroid. We hypothesized that retinal vascular calibre prior to anti VEGF treatment for neovascular AMD could act as a surrogate marker for the degree of choroidal ischaemia and thus potentially predict treatment response by indicating the degree of underlying disease severity.

Methods

In order to assess the influence of retinal vascular calibre on treatment outcome, we obtained colour fundus photographs of patients at the time of diagnosis and subsequent 3-monthly intervals, to determine the CRAE and CRVE. The influence of baseline measurements of CRAE and CRAE were correlated with anti-VEGF treatment outcome at 12 months. In the second analysis, the influence of anti-VEGF treatment on retinal vascular calibre was studied.

Results

In a group of 88 patients, we found that after accounting for age, gender, size of choroidal neovascularization (CNV) and number of injections, patients who deteriorated in visual acuity at 12 months had significantly larger baseline CRVE, 243.10µm (95%CI, 227.01, 259.19), compared to those who were stable, 214.30µm (95%CI 205.79, 222.81) and those who improved, 215.26µm (95%CI 204.69, 225.84), p=0.007. Baseline CRAE did not differ significantly in eyes whose vision deteriorated, 150.12µm (95%CI 140.67, 159.57), compared to those remaining stable, 143.64µm (95%CI 138.64, 148.63), or gaining vision 142.92µm (95%CI 136.71, 149.13), p=0.69. CMT change from baseline to 12 months was not associated with either CRVE change or CRAE change.

Discussion

The specific underlying mechanism for our observations of wide retinal venular calibre being associated with poorer visual outcome is unclear, but it is possible that the degree of retinal venular dilatation at diagnosis of CNV may be a marker of choroidal ischaemia and thus disease severity. In keeping with this hypothesis, retinal venular dilatation has been described as a predictive factor in the development of cardiovascular diseases such as stroke and coronary heart disease, independent of traditional cardiovascular risk factors. Additionally, retinal venular widening has been demonstrated in primates injected with vascular endothelial growth factor.[108] As such, our observations of widening of retinal venules and poorer outcome of treatment with anti- VEGF treatment are interesting and may represent either more severe disease at baseline, with increased VEGF levels in the eye or, alternatively, a marker of end-stage disease, with neural disorganization or fibrosis, predicting poorer response to anti-VEGF therapy.

5. TREATMENT REGIMEN

The results of this work was published in the following journals; Retina, Eye and clinical and Experimental Ophthalmology.

Lessons learnt inform our approach to new antivascular endothelial growth factor treatments for neovascular age-related macular degeneration. **Wickremasinghe S**, Abedi F, Sandhu SS, Guymer R. Clin Experiment Ophthalmol. 2013 Nov;41(8):723-6

Letter to Editor

Visual acuity outcomes of an Inject and Extend Protocol of Anti-VEGF Treatment for Neovascular Age Related Macular Degeneration. Abedi 2, **Wickremasinghe SS**, Islam FA, Guymer RH. Retina 2014 Aug;34(8):1531-8.

PURPOSE: To evaluate 2-year visual acuity outcome of a treat-and-extend protocol of anti-vascular endothelial growth factor treatment in age-related macular degeneration.

METHODS: In this prospective cohort study, 120 age-related macular degeneration patients with choroidal neovascularization received 3 initial monthly ranibizumab or bevacizumab injections; monthly injections were continued until there was no choroidal neovascularization activity (subretinal/ intraretinal fluid, loss of >5 letters, or persistent/recurrent retinal hemorrhage). When there was no choroidal neovascularization activity, the interval to the next visit/injection was extended by 2 weeks to a maximum of 12 weeks. In the presence of choroidal neovascularization activity, this interval was shortened by 2 weeks. Main outcome measures included the percentage losing <15 letters and the mean visual acuity change after 12 months and 24 months.

RESULTS: Mean baseline visual acuity was 51.2 ± 12.1 Early Treatment Diabetic Retinopathy Study scores. Mean visual acuity change from baseline was +9.5 ± 10.9 and +8.0 ± 12.9 letters after 12 months and 24 months, respectively, with, on average, 8.6 ± 1.1 visits/injections in the first year and 5.6 ± 2.0 in the second year. After 12 months and 24 months, 97.5% and 95.0% of patients, respectively, lost <15 letters.

CONCLUSION: The "inject-and-extend" protocol-with fewer injections and visits-delivered outcomes comparable to those of the pivotal clinical trials of monthly ranibizumab.

Ranibizumab treatment for neovascular age-related macular degeneration: from randomized trials to clinical practice.

Michalova K, **Wickremasinghe SS**, Tan TH, Chang A, Harper CA, Downie JA, Hunyor AP, Guymer RH. Eye (Lond). 2009 Aug;23(8):1633-40.

BACKGROUND: Although randomized clinical trials (ANCHOR and MARINA) have shown excellent results of ranibizumab treatment in patients with neovascular age-related macular degeneration (AMD), it is unclear whether such an outcome is achievable in daily practice. We evaluated the results of ranibizumab treatment for neovascular AMD in clinical practice in Australia.

METHODS: A retrospective chart review of patients in four practices injected with ranibizumab in 2006 for AMD. Patients who had been diagnosed with subfoveal choroidal neovascular membrane in the preceding 6 months and had completed at least 6 months follow-up were enrolled. No standard treatment protocols were required. The main outcome measure was visual acuity (VA) at 6 and 12 months.
RESULTS: A total of 158 patients fulfilled the entry criteria. The mean baseline VA (decimal) was 0.35+/-0.21 (Snellen equivalent 6/17). At 6 months, the mean VA improved to 0.46+/-0.27 (6/13) and remained stable until month 12 (0.48+/-0.30). The improvement in VA between baseline and months 6 and 12 was statistically significant (P<0.0001). Both the mean and the median number of injections were four in the first 6 months and nine at 12 months. VA results were comparable with those of the ANCHOR and MARINA trials, and were achieved with a lower number of injections (P<0.0001).
CONCLUSION: VA results achieved in daily clinical practice using ranibizumab for neovascular AMD are similar to large prospective randomized trials.

As a result, of the desire to limit anti-VEGF injection frequency, many clinicians tried varying the protocol in an attempt to reduce the treatment burden (reduced number of injections and follow up visits required), whilst achieving the same visual results as the trials mandating rigorous monthly injections. This was the case in the retina clinics at the Royal Victorian Eye and Ear Hospital (RVEEH), the first public hospital in Australia to offer this treatment.

Methods

1. During our early experience with the use of anti-VEGF injections (2006-7), after a

loading phase of three monthly injections, a *pro re nata* (PRN) treatment regimen was mostly used, whereby, the decision to re-treat was made solely by the clinicians, depending upon the patients response to the preceding treatment at each subsequent visit. This was based upon the ophthalmic examination findings such as new or persistent haemorrhage, loss of visual acuity OCT findings, such as an increase in central macular thickness or the presence of either IRF or SRF. Four to six weekly follow up was maintained throughout this period.

2. After 2008, we widely adopted a treat and extend treatment regimen at RVEEH. In the treat and extend strategy, following stabilisation of the disease with initial 'loading dose' injections, patients are treated periodically, even if the disease remains inactive, but the treatment free period is gradually extended following each injection. This has the advantage of finding the optimal dosing period for each patient, without subjecting the patient to significant recurrence of disease. Where there is a recurrence in the symptoms or signs of exudation, the treatment interval is shortened accordingly to the last stable interval.

In summary, the treat and extend strategy utilizes three initial monthly injections. Following the third injection, at each subsequent visit, dilated fundal examination and OCT assessment of the study eyes is used to determine the presence of residual activity within the neovascular membrane. If BCVA is stable (no loss of >5 letters), with no signs of persistent or new hemorrhage on dilated fundal examination and the OCT retinal image is free of subretinal fluid (SRF) or intraretinal oedema/cysts (IRF), an injection is given and the interval to the next visit is extended by two weeks, to a maximum of 12 weeks. In the extension phase, in the event of a drop in BCVA of > 5 letters, recurrent disease (new haemorrhage, or recurrent SRF/ IRF on OCT), an

anti-VEGF treatment is administered and the interval to the next injection is shortened by 2 weeks, back to an interval where the patient had been stable.

If there is persistent SRF or IRF on OCT or signs of residual neovascular activity on clinical examination (residual subretinal blood) after the three initial injections, monthly treatment is continued until the OCT is deemed to be free of fluid and the BCVA is stable. At this point, an extension in the follow up period is considered (as described above).

The advantages of this strategy is that it minimises the treatments compared to monthly injections, and number of visits compared to PRN, but yet reduces the potential length of a recurrences and the ensuing retinal damage caused by frequent re-accumulation of fluid that can occur in an 'as required' dosing strategy, where in some cases the vision cannot be recovered despite the re-institution of frequent injections.

3. Other treatment strategies have been successfully used such as the flexible dosing schedule used in the Prospective OCT Imaging of Patients with nAMD Treated with intraocular Ranibizumab (PrONTO) study. This method utilized OCT-measured central retinal thickness measurements and visual acuity to guide anti-VEGF re-treatment.[109] In the real world treatment of neovascular AMD, varying regimens are used. We assessed the treatment outcomes in the four private practices where patients were treated with ranibizumab. No standard treatment protocols were required. The main outcome measure was visual acuity at 6 and 12 months.

Results

1. An early audit of our results showed disappointing visual outcomes compared to the pivotal trials, with change in acuity of +4.0 ± 11.9, +1.6 ± 12.6, -3.4 ± 15.5 and -6.9 ± 17.8 letters, at 3,6, 9 and 12 months respectively. These disappointing results were achieved with the mean number of 4.2 ± 2.0 injections in the first 12 months, an extremely low number of injections and in part reflects that some cases, were chronic cases where treatment was offered without any real expectation of improved outcomes, but was offered just in case, then when no benefit was gained the treatment was ceased after just a few months.

2. Auditing the visual outcomes from the RVEEH clinics up until the end of December 2011, we collected audit data on 459 eyes of 399 patients who had commenced anti VEGF treatment at the hospital between 2006 and Dec 2011 where 289 (63%) of eyes were still under active follow up at the RVEEH clinic, and 238 (82.4%) of these eyes were continuing to receive anti-VEGF treatment. Mean change in VA for each commencement year cohort over the course of the study showed that the VA outcome of patients who commenced treatment after 2008 was better than that of those commencing in the 2006 and 2007 cohorts (Figure 13).

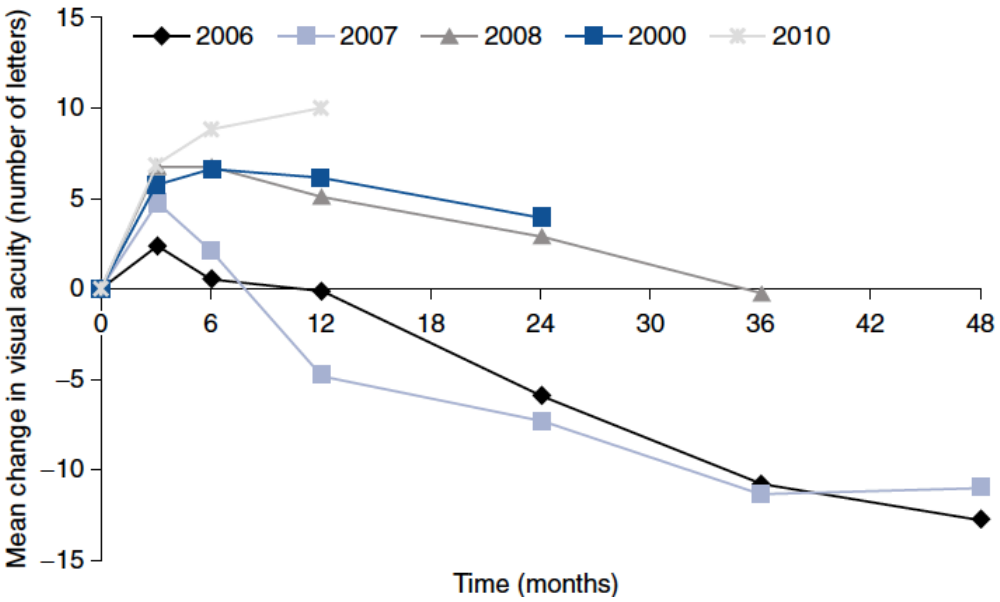


Figure 13. Change in visual acuity over time characterised by the year of commencing anti-VEGF treatment.

The improved VA outcome was achieved with increasing numbers of injections, with the mean number of injections increasing steadily after 2008, with patients who commenced anti-VEGF treatment in; 2006 receiving 3.9 ± 2.7 in the first year of treatment. In subsequent years, patients commencing treatment received 4.2 ± 2.0, injections in 2007, 5.1 ± 2.3, in 2008 and 6.7 ± 2.5, in 2009. In 2010, 7.5 ± 2.1 injections were administered. Thus although desirable to reduce the number of injections given to patients, it is not possible to greatly reduce injection numbers in the first year of treatment and expect to maintain the overall improved visual outcomes. With strict re-treatment criteria however, using a well defined treat and extend strategy, results similar to the pivotal studies with a reduced number of treatments can be achieved safely in most patients.

3. In four private practices throughout Victoria and New South Wales, Australia, the visual outcomes of patients undergoing ranibizumab treatment for neovascular AMD were assessed retrospectively. A total of 158 patients fulfilled the entry criteria, with subfoveal choroidal neovascularisation being confirmed on fundus fluorescein angiography. The mean baseline VA (decimal) was 0.35± 0.21 (Snellen equivalent 6/17). At 6 months, the mean VA improved to 0.46± 0.27 (6/13) and remained stable until month 12 (0.48± 0.30). The improvement in acuity between baseline and months 6 and 12 was statistically significant ($p<0.0001$). Both the mean and the median number of injections were four in the first 6 months and nine at 12 months. Acuity results were comparable with those of the ANCHOR and MARINA trials, and were achieved with a lower number of injections ($p<0.0001$).

Discussion

Although desirable to reduce the number of injections given to patients, it is not possible to greatly reduce injection numbers in the first year of treatment and expect to maintain the overall improved visual outcomes. With strict re-treatment criteria however, using a well defined treat and extend strategy, results similar to the pivotal studies with a reduced number of treatments can be achieved safely in most patients.

'As required' dosing can also be a reasonable option as long as monthly follow up is maintained, as described in the CATT, SUSTAIN and PrONTO studies. With careful clinical examination and OCT imaging, the visual results may approach those of the pivotal studies, but with the use of significantly less injections. Ultimately however careful patient selection of who will benefit from treatment, with the knowledge that some patients have irreversible end-stage disease (at presentation) may optimise outcomes and limit unnecessary treatment.

Another important consideration is duration of treatment. The importance of ongoing injections is clearly demonstrated in our audit. Even in 2008 and 2009 where visual outcome in the first 12 months was good, in subsequent years the visual outcomes were not so impressive, with a drift back towards baseline acuity. This has also been shown in the HORIZON and 7-UP extension studies of the pivotal ANCHOR and MARINA patient, where at 4 and 7 years respectively, there was deterioration in vision that was consistent with a reduced treatment frequency.[110,111]

6. COMPLICATIONS OF ANTI-VEGF INJECTIONS

Acute intraocular inflammation after intravitreous injections of bevacizumab for treatment of neovascular age-related macular degeneration. **Wickremasinghe SS**, Michalova K, Gilhotra J, Guymer RH, Harper CA, Wong TY, Qureshi S.

Ophthalmology. 2008 Nov;115(11):1911-5

PURPOSE: Bevacizumab is an inhibitor of vascular endothelial growth factor widely used as an "off-label" treatment of neovascular age-related macular degeneration (AMD), despite the lack of clinical trial data on efficacy or safety of this drug. We describe acute intraocular inflammation after intravitreous injection of bevacizumab for the treatment of neovascular AMD.
DESIGN: A retrospective case series.
PARTICIPANTS: Patients with neovascular AMD treated with intravitreous injection of bevacizumab from clinical practices in 2 states (Victoria and South Australia) in Australia.
METHODS: We retrospectively reviewed cases of acute intraocular inflammation after intravitreous injection of bevacizumab for the treatment of neovascular AMD.
MAIN OUTCOME MEASURES: The detection and description of inflammation in a large cohort of patients.
RESULTS: There were 14 cases (11 women and 3 men), from a total of 1278 injections given. The mean age of patients was 83.7 years (range, 74-98). The majority had a prior injection of bevacizumab, with a mean number of injections of 2.7 (range, 1-6). Most patients presented within 24 hours of intravitreous injection, with rapid reduction in vision, but minimal discomfort. There were associated signs of ocular inflammation in the anterior and posterior segments of the eye. Visual acuity at presentation was substantially reduced compared with the preinjection acuity, although the vision rapidly improved with treatment over a period of 7-25 days toward preinjection visual acuity.
CONCLUSIONS: Intravitreous injection of bevacizumab for the treatment of neovascular AMD may be associated with acute intraocular inflammation. Differentiation from infectious endophthalmitis is important for appropriate management of this condition.

Reports of acute inflammation following the use of intravitreal anti-VEGF agents have previously been reported. During a period of 12 months we studied the characteristics of patients with inflammation following injections of bevacizumab.

Although uncommon, complications associated with anti-VEGF use have been documented. An international online 'survey' on the safety of bevacizumab noted only a very small minority of patients developed any complications following intravitreous injection, with no specific adverse event exceeding 0.21%.[112] A few case reports have reported mild anterior uveitis and endophthalmitis following injection and the ANCHOR and MARINA trials noted between 1.3% and 2.2% of patients had moderate to severe inflammation following intravitreous injection.[1,2]

In our practice at RVEEH, we noted occasional patients with severe inflammation following intravitreal injections. We therefore retrospectively collected a series of patients presenting with inflammation following intravitreal anti-VEGF injections in order to determine if there were any commonly associated features. Over a12 month period 1278 intravitreous injections of bevacizumab were given at 6 practices, within 2 states of Australia. Of these injections, 19 cases were associated with acute inflammation (1.49%). Mostly the presentation was acute, with most patients presenting within a day of injection with rapid reduction in vision, but minimal discomfort. There were associated signs of moderate to severe ocular inflammation in the anterior and posterior segments of the eye. The visual acuity at presentation was substantially reduced compared to the pre-injection acuity, although the vision rapidly improved with treatment over a period of 7-25 days toward pre-injection visual acuity. The number of prior injections did not appear to correlate with the degree of anterior chamber or vitreous inflammation. The time from injection to presentation with inflammation did not appear to affect the extent of visual recovery, although the

time to recovery of vision was slightly longer in those patients presenting ≥ 2 days after injection (14.8± 6.1 days as compared to 10.8± 3.3 days). This inflammation has a particular clinical presentation and although differentiation of severe inflammation from infectious endophthalmitis can be difficult, its early presentation and predominant anterior chamber involvement are specific clues.

Knowledge of this presentation, with floaters and minimal pain may help to differentiate inflammation from sight threatening endophthalmitis. Endophthalmitis is usually associated with pain and progressive dimming of vision rather than floaters.

Interestingly, most of our patients recovered vision towards their pre-injection levels. Again this would be unusual in cases of endophthalmitis.

We speculated several possible causes for inflammation in our cohort, some more likely than others. Although improbable, it is possible that contamination of the bevacizumab with bacterial endotoxin at the time of individual aliquot preparation may have occurred. Thus, following intravitreous injection a reaction analogous to the toxic anterior segment syndrome (TASS) following cataract surgery may have taken place. Bacterial endotoxin is a frequent and recalcitrant contaminant of antibody preparations during the production phase of the pharmaceutical agent. Preparations of bevacizumab that are specifically prepared for intravenous use may contain traces of endotoxin at levels that incite intravitreous inflammation even though they are of no significance when the drug is administered systemically. Since most eyes had had previous injections, it is possible that the eye may have mounted an immune anti-idiotypic response to the anti-VEGF antibody. Larger molecules with Fc (constant) fragments as well as the antibody binding (Fab) fragments are more immunogenic than those with the antibody binding fragment alone. It is possible that with increased sensitization (after multiple injections), there may be an increased immune response.

Finally, storage of the drug may have been compromised. The manufacturer's guidelines state that bevacizumab vials must be refrigerated at 2°C to 8°C (36°F to 46°F), should be protected from light and should be stored in the original carton until time of use. It is also recommended that bevacizumab is used within 8 hours of opening. (Avastin prescribing information. San Francisco, CA: Genentech, Inc; December 2004. Genentech, Inc.) Whether any variation in storage temperature happened in our series is unknown, but theoretically some degradation of the drug may have occurred and rendered the injection more immunogenic.

7. RELATIVE IMPACT OF PREDICTORS OF OUTCOME

We recently reviewed the literature on the possible influences on anti-VEGF treatment outcome in patients with neovascular AMD.

Predictors of anti-VEGF treatment response in neovascular age-related macular degeneration.

Finger RP, **Wickremasinghe SS**, Baird PN, Guymer RH. Surv Ophthalmol. 2014;59(1):1-18.

Review

Currently available evidence on predictors of anti-vascular endothelial growth factor (VEGF) treatment response in neovascular age-related macular degeneration was reviewed. No meta-analysis of results is possible because of a lack of controlled and randomized trials, varying treatment regimes and outcome measures used, as well as suboptimal reporting. For genetic factors, most evidence to date has been generated for single nucleotide polymorphisms (SNPs) in the complement factor H (CFH), and VEGF-A genes. Just under half of the SNPs assessed in the CFH gene and 15% of the SNPs assessed in the VEGF gene were found to be associated with visual outcomes or the number of injections required during follow-up. Some evidence suggests association of worse treatment outcomes as well as a younger age at treatment onset with an increasing number of risk alleles in known risk genes (CFH and ARMS2/HTRA1) and polymorphisms in the VEGF-A gene. Clinical factors such as higher age, a better visual acuity (VA), a larger choroidal neovascularization (CNV) lesion at baseline, and a delay between symptom onset and initiation of treatment of more than 3 weeks also impact outcomes. Conversely, a worse acuity at baseline predicted more gain in vision. Overall, patients presenting with good acuity at baseline were more likely to have good VA at follow up, but the gain afforded by treatment was impacted by a ceiling effect. Most available evidence suggests a strong association of clinical factors such as age, baseline VA, and CNV lesion size with anti-VEGF treatment outcomes. No behavioral factors such as smoking influence treatment outcomes. Based on the studies conducted so far, the evidence suggests that underlying genotype of known AMD risk associated genes or of the VEGF-A gene have a limited effect, whereas presenting clinical factors appear to be more important in determining treatment outcomes.

Predictors of treatment outcomes are necessary to not only plan treatment in the most cost-effective way, inform the patient about his or her individual prognosis, but can also be used to select the most appropriate treatment and treatment protocol. In this systematic review of the literature we were not able to perform a meta-analysis of the results because of a lack of controlled and randomized trials, varying treatment regimes and outcome measures used, as well as suboptimal reporting.

Based on the studies conducted so far, the evidence suggests that underlying genotype of known AMD risk associated genes or of the *VEGF-A* gene have a limited effect, whereas presenting clinical factors appear to be more important in determining treatment outcomes.

Clinical factors such as higher age, a better visual acuity, larger choroidal neovascularization lesion at baseline and a delay between symptom onset and initiation of treatment of more than three weeks also impacted on visual outcomes. Conversely, a worse acuity at baseline predicted more gain in vision most likely as a 'floor' effect. Overall, patients presenting with good acuity at baseline were more likely to have good vision at follow up, but the gain afforded by treatment was impacted by a 'ceiling effect'.

Genetic factors may be associated with anti-VEGF treatment outcomes, whereby a larger number of risk alleles in known AMD risk genes predict worse treatment outcomes and earlier treatment onset in life. However, the effect of these known genetic factors currently appears to be small based on the reviewed studies. With subsequent, larger and thus better-powered studies, the size and nature of an influence of genetic factors on treatment outcomes in neovascular AMD may become more evident. An alternative possibility is that other genes so far not identified predict outcome in a much stronger manner but this will rely on a genome wide pharmacogenetic approach in identifying them.

FUTURE DIRECTIONS

Of the various cytokine families that play roles in the development, maintenance, and remodelling of microcirculation, the VEGF family is the most studied, although several other cytokines, inflammatory cells and growth factors have been implicated in the pathogenesis of AMD and may play a role in the outcomes associated with neovascular complications of the disease.

Anti-VEGF therapy has been shown to influence the intraocular microenvironment beyond the effect on VEGF suppression.[113] As a result, it is possible that anti-VEGF monotherapy is adequate, in the majority of people to suppress neovascular activity and thus maintain vision. In eyes with poor response to anti-VEGF treatment however, it is possible that either other mediators, such as those involved in inflammation may be involved in the development or maintenance of neovascular tissue. Inadequate suppression of these other mediators may explain the progressive deterioration of vision or persistent IRF or SRF despite anti-VEGF treatment. Interleukin 6 and 8 have been shown to be associated with macular volume and thickness in eyes with neovascular AMD.[113] Other growth factors such as PDGF and pigment epithelial growth factor (PEDF) have also been implicated in the development of neovascularisation.[114] PDGF ligands and receptors are widespread on proliferative retinal membranes of patients with proliferative retinopathies and PDGF acts as a survival factor for retinal pericytes. Pericytes provide stabilization to the newly synthesized vessels and also inhibit the penetration of anti-VEGF agents to the vessel surfaces, thus limiting their effect.[115] As a result combination treatments may be required in these eyes in the hope of optimizing outcomes.

An alternative hypothesis for the differential response to anti-VEGF treatment may relate to inadequate/ over-suppression VEGF suppression with current agents. Although VEGF levels are rapidly reduced following treatment, it is possible that extremely elevated levels are associated with a poorer response than where VEGF concentrations are closer to the normal range. VEGF also has physiological functions

in the eye. Reduction of VEGF to below the physiological range, may have deleterious effects. Heiduschka et al, demonstrated a reduction in choriocapillaris fenestrations following anti-VEGF injections in primates, thus potentially limiting the nutrition of the RPE and outer retina.[116] In the CATT study, the use of anti-VEGF agents was associated with the development of geographic atrophy. Prior knowledge of VEGF levels in the vitreous/ aqueous may thus help to influence the treatment regime. Thus in some patients prior knowledge may mandate more regular treatment, rather than using variable dosing strategies, whilst in others, limiting anti-VEGF to a PRN dosing may be more appropriate.

Some preliminary work in this area has been published. In a small study by Funk et al., it was found that that aqueous concentration of VEGF was elevated in eyes with neovascular AMD, although PDGF was reduced. Furthermore, a significant decrease of VEGF was observed after a single intravitreal injection of ranibizumab along with significant changes in visual acuity and central retinal thickness. During follow-up with a flexible regimen, a correlation was identified between increased VEGF levels and persistent or recurrent macular edema.[114]

In our review of the literature, the influence of pharmacogenetics on treatment response in neovascular AMD has been inconclusive, most studies have been small and have yielded varying results with mixed results associations between individuals' genetic make up and their response to treatment. Thus alternative methods to determine possible associations may be more appropriate. Genome-wide association (GWA) studies is a genetic analysis method that searches the whole genome for small variations (single nucleotide polymorphisms), which occur more frequently in people with a particular disease than in people without the disease. If one type of the variant (one allele) is more frequent in people with the disease, the SNP is said to be

"associated" with the disease. The associated SNPs are then considered to mark a region of the human genome that influences the risk of disease. In contrast to methods that specifically test one or a few genetic regions, the GWA studies investigate the entire genome. The approach is therefore said to be non-candidate-driven, in contrast to gene-specific candidate-driven studies. GWA studies identify SNPs and other variants in DNA which are associated with a disease, but cannot on their own specify which genes are causal.[117]

One study to date conducted a genome wide association study (GWAS) on 65 patients and reported an association of the CFH gene with less improvement, the C3 gene with a reduced central retinal thickness (CRT) on OCT and an association between CFH and VEGFA genes and a smaller number of injections during follow up (12 months).[48] The GWAS identified one additional gene, the fms-related tyrosine kinase 1 gene (FLT1), which is the receptor to VEGF, to be associated with persistent leakage on fluorescein angiography (FA) and a smaller number of injections over 12 months.[48]

With respect to imaging, our work utilised a time-domain OCT. This technology is not as precise as either spectral domain or swept source OCT systems. It is possible that we were not able to fully identify residual or recurrent fluid (as a marker of neovascular activity) in our studies, which may have influenced our results. Another more definable feature present on spectral domain OCT, is the assessment of integrity of the outer retina (the so called IS/OS line or ellipsoid layer of the photoreceptors). This as well as the external limiting membrane has been found to correlate with visual acuity in patients with diabetic macular oedema, retinal vein occlusion and neovascular AMD. Thus now that spectral domain OCT is more widely available, it may be appropriate to repeat our work.

Additionally, other anatomical features can now be readily imaged, such as fundus autofluoresence and enhance depth imaging of the choroid. Fundus autofluoresence assesses the integrity of the outer retina and retinal pigment epithelium using a blue or green laser light source. Areas of hyper-autofluoresence are thought to be associated with “stressed” retina and RPE, whilst hypo-autofluoresence is associated with loss of function. Thus these features when present at baseline may also be able to predict poorer vision outcome following treatment. Enhanced depth imaging (EDI) of the choroid using spectral domain OCT may also be a useful marker of disease severity. Previous studies have identified that individual with AMD have reduced choroidal thickness and thus, assessment at baseline may allow prediction of treatment response to anti-VEGF drugs.

CONCLUSIONS

We have identified features such as the presence of intraretinal fluid on OCT, delay in initiating treatment following the first symptoms of CNV, larger retinal venular calibre and inadequate treatment in predicting a poorer response to treatment. At present the only modifiable risk factors are reducing the delay in instituting treatment from the first symptoms of choroidal neovascularisation and ensuring optimal dosing of anti-VEGF injections.

Education of patients and primary health care physicians/ optometrists in the symptoms of this devastating disease will allow more rapid diagnosis and referral for treatment and thus improve outcomes.

With respect to dosing, we have shown that a 'treat and extend' regimen is effective at improving vision and reducing injection frequency. Other methods such as pro re nata dosing are also effective, although both strategies require accurate monitoring with OCT. We have shown that it is important to eliminate fluid from the macula, particularly IRF and thus correct interpretation of OCT images is important to decide the timing and need for further treatment.

Other factors such as genetic make are not currently modifiable. Further work at understanding why genes such as *HTRA1/ARMS2*, *VEGF* and *APOE* influence treatment response is needed, since it may be possible to modify the protein product of the genes and thus influence response if not the genes themselves. Further knowledge may also help us to understand how best to individualize a treatment strategies based on genetic polymorphisms. Finally, identification of the genes influencing treatment response to anti-VEGF injections may also be important since, in the future modification of the AMD susceptible genes, to either prevent the development of neovascular AMD or optimise patient response to treatment may be possible.

Imaging of features such as retinal vasculature prior to initiation of treatment may also help to instruct physicians about the likely course of treatment and may also help to give an idea of prognosis to patients. Prior knowledge of these characteristics may also allow individualisation of treatment regimens, such as more intensive treatment or combination treatments, in order to optimise outcomes.

Individualising treatment regimes is ideal for patients such that eyes requiring more intensive treatment can be identified early to optimise outcomes. Similarly if identified early, eyes with less aggressive disease may avoid having unnecessary injection, whilst still achieving excellent results. Currently Phase IV studies are underway to assess if the aim to completely dry the retina treatment appropriate in all patients and thus assess whether less aggressive treatment may be appropriate in certain conditions despite the presence of persistent fluid. The FLUID study (Comparison of Treatment Regimens Using Ranibizumab: Intensive (Resolution of Intra- and Sub-retinal Fluid) vs. Relaxed (Resolution of Primarily Intra-retinal Fluid) Treatment. NCT01972789 Novartis Pharmaceuticals), is a Phase IV study utilising ranibizumab in a 'Treat and Extend' regime in patients with neovascular AMD. This randomised study will assess the visual outcome at 24 months in the "intensively" treated group (aim to dry retina completely before any extension) or the "relaxed" group (aim to extend between visits unless there is intraretinal fluid or no increase in subretinal fluid).

Ultimately anti-VEGF treatment is costly. In total, since it was listed in 2007, Ranibizumab (Lucentis) has cost the Australian taxpayer more than $1.38 billion and we have seen a halving of the rate of blindness as a result. However treatment with anti VEGF does not address the underlying disease and so treatment is for a prolonged, if not life long, time frame. Thus it is imperative to greater understand the underlying drivers that cause progression of early AMD to neovascular AMD, in order to reduce the incidence of CNV. Also factors that influence response must be fully

characterised, in the hope of maintaining good visual outcomes but also reducing the treatment burden to the patient and also to society.

Over the next few years the development of new anti-VEGF drugs as well as combination treatments may improve visual outcomes in this previously blinding disease. Such treatments are currently in Phase II and III development and hopefully the results will allow physicians greater options in improving patient outcomes.

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APPENDIX

Publications by chapter

Clinical characteristics

1. Delay to treatment and visual outcomes in patients treated with anti-vascular endothelial growth factor for age-related macular degeneration. Lim JH, Wickremasinghe SS, Xie J, Chauhan DS, Baird PN, Robman LD, Hageman G, Guymer RH. Am J Ophthalmol. 2012;153(4):678-86, 686.

OCT characteristics

2. Predictors of AMD treatment response. Wickremasinghe SS, Sandhu SS, Busija L, Lim J, Chauhan DS, Guymer RH. Ophthalmology. 2012;119(11):2413-2414.

Genetic influences

3. Genetic influences on the outcome of anti-vascular endothelial growth factor treatment in neovascular age-related macular degeneration. Abedi F, **Wickremasinghe S**, Richardson AJ, Islam AF, Guymer RH, Baird PN. Ophthalmology. 2013;120(8):1641-8.

4. Variants in the VEGFA gene and treatment outcome after anti-VEGF treatment for neovascular age-related macular degeneration. Abedi F, **Wickremasinghe S**, Richardson AJ, Makalic E, Schmidt DF, Sandhu SS, Baird PN, Guymer RH. Ophthalmology. 2013;120(1):115-21.

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Treatment Regimen

7. Lessons learnt inform our approach to new anti Anti-VEGF Treatments for Neovascular Age-related Macular Degeneration. **Wickremasinghe S**, Abedi F, Sandhu SS, Guymer R. Clin Experiment Ophthalmol. 2013;41: 723-6

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Complications

10. Acute intraocular inflammation after intravitreous injections of bevacizumab for treatment of neovascular age-related macular degeneration. **Wickremasinghe SS**, Michalova K, Gilhotra J, Guymer RH, Harper CA, Wong TY, Qureshi S. Ophthalmology. 2008 Nov;115(11):1911-5

Relative impact of predictors of outcome

11. Predictors of anti-VEGF treatment response in neovascular age-related macular degeneration. Finger RP, **Wickremasinghe SS**, Baird PN, Guymer RH. Surv Ophthalmol. 2014;59(1):1-18.

Delay to Treatment and Visual Outcomes in Patients Treated With Anti-Vascular Endothelial Growth Factor for Age-Related Macular Degeneration

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• PURPOSE: To investigate the potential influences that affect visual acuity (VA) outcome in a clinic-based cohort of age-related macular degeneration (AMD) patients undergoing anti–vascular endothelial growth factor (anti-VEGF) treatment for choroidal neovascularization.

• DESIGN: Prospective interventional case series.

• METHODS: Patients with subfoveal choroidal neovascularization (CNV) secondary to AMD were prospectively recruited. A detailed questionnaire was given to patients at time of enrollment, to collect information relating to demographics, history of visual symptoms, visual acuity (VA), and treatment scheduling. Delay from symptoms to treatment ("Treatment delay") was measured in terms of weeks and analyzed in tertiles. Information pertaining to treatment outcomes was collected over a 6-month period.

• RESULTS: One hundred eighty-five eyes of 185 patients were recruited into the study. Longer delay from first symptoms suggestive of CNV to first injection was a significant predictor (P = .015) of poorer treatment outcome, when controlling for age, sex, and baseline VA. Patients with a delay in treatment of 21 weeks or more compared to a delay of 7 weeks or less had an odds ratio of 2.62 (1.20, 5.68) for worsening vision after treatment.

• CONCLUSIONS: Patients experiencing a longer delay between their first symptoms of CNV and their first anti-VEGF treatment have a significantly lower chance of improving vision at 6 months following anti-VEGF therapy. It is critical that this information reach those at potential vision loss from AMD, in order that prompt treatment may be instituted, to maximize the benefits of anti-VEGF treatment. (Am J Ophthalmol 2012;153: 678–686. © 2012 by Elsevier Inc. All rights reserved.)

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RANIBIZUMAB (LUCENTIS; GENENTECH INC, SOUTH San Francisco, California, USA) and bevacizumab (Avastin; Genentech Inc) are anti–vascular endothelial growth factor (anti-VEGF) drugs that have improved the treatment of neovascular age-related macular degeneration (AMD).[1–7] However, between 10% and 15% of patients treated in the pivotal MARINA (Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD) and ANCHOR (Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in AMD) randomized controlled trials did not experience the visual acuity (VA) benefits enjoyed by most participants but instead continued to lose vision despite treatment.[1–3] Most of the acuity increase occurred in the first 3 months of treatment; however, in those patients who responded poorly, vision failed to improve from the outset, with continued losses noted during the course of the treatment regimen. To date, there is no satisfactory explanation as to why some patients with neovascular AMD respond poorly to treatment.

Ranibizumab and bevacizumab are effective for the treatment of subfoveal choroidal neovascularization (CNV),[1–3,8] and it seems intuitive that the sooner the neovascular process is arrested, the less damage would be impacted on the retina and the sooner anatomic integrity of this structure would be restored, resulting in better outcome. Data from the MARINA and ANCHOR studies do not show any detrimental effect on outcome with delay to treatment, from the date of first angiographic diagnosis of CNV.[9,10] This delay is often minimal, as once the patient has a confirmed lesion on angiography the decision to treat is usually swift, with little delay in its implementation. While some delay can occur because of bureaucratic processing of authority to prescribe expensive anti-VEGF treatment, or in cases of stable occult lesions where the decision to treat is often delayed until signs of progression appear, the main delay is often from the time of the first symptom suggestive of CNV (metamorphopsia, central blur, central scotoma) to presentation to the treating ophthalmologist. However, this time interval is imprecise and is often not asked for in any detail from patients, and, as such, is never presented in results of clinical trials. Despite this, a number of attempts have been made to document this period retrospec-

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tively.[11,12] Retrospective evaluation can be difficult, however, since the onset of symptoms often cannot readily be determined without detailed specific questioning of the patient by a trained clinician. Additionally, the timing of symptom awareness is likely to differ, depending on the visual acuity in the other eye. Yet it is this period, from first onset of symptoms to the eventual treatment, that is likely to be quite variable and in some cases prolonged. Thus, although it is less accurate than the angiographic diagnosis, we thought it crucial to investigate to gain a better understanding of the influences on outcome to treatment, particularly as this time interval would be accessible to modification. We hypothesize that this time interval varies significantly among patients and, if shortened, is likely to have a profound effect on treatment outcome. We undertook a study to prospectively determine influences on treatment outcome with anti-VEGF drugs in AMD, and report here on the influence delay from first symptoms suggestive of CNV has on outcome, as well as the delay from the angiographically confirmed CNV to VA outcome.

This study was clinic-based with individual consultants planning their treatment schedules. In this study we chose to look at the 6-month VA outcomes, as from several pivotal randomized controlled trials[3,4] it is clear that in the vast majority of cases the visual acuity increase was seen in the first 3 months of treatment, and in those patients who responded poorly, vision failed to improve from the outset, with continued losses noted early during treatment. Investigating VA outcomes at a later time point introduces the opportunity for other variables, such as injection timing and number and missed appointments, to play a role that is minimized at the shorter review time. Thus we felt it more useful to investigate an early time point outcome to address the specific issue of delay to first treatment.

METHODS

• STUDY DESIGN AND ELIGIBILITY: Study patients were recruited consecutively between August 1, 2007 and December 31, 2008 from the Medical Retina Clinic at the Royal Victorian Eye and Ear Hospital and the private rooms of the Vision Retinal Institute Eastern, in Melbourne. All patients were over the age of 50 years and were diagnosed with subfoveal CNV (subfoveal lesions included those juxtafoveal lesions that could not be safely treated with either thermal laser or photodynamic therapy, without involvement of the center of the fovea) secondary to AMD. Classic or occult leakage was included as long as the area of leakage extended under the fovea. Patients with retinal angiomatous proliferation were classified as having occult lesions, as were patients with pigment epithelial detachment. Such patients were only recruited if there was a component of occult choroidal neovascularization suggested by a notch or nonuniform filling. Lesions with significant sero-sanguinous detachments were excluded on the basis of indocyanine green angiography if the diagnosis of idiopathic polypoidal choroidal vasculopathy was suspected.

The main exclusion criteria were: 1) diagnosis of CNV secondary to other eye conditions; 2) laser photocoagulation or photodynamic therapy prior to anti-VEGF injections; and 3) non-white ancestry.

• DATA COLLECTION AND FOLLOW-UP: This study was designed to investigate all parameters that might influence treatment outcomes following anti-VEGF treatment. A questionnaire/information sheet was employed to collect demographic information and medical history as well as history of visual symptoms, VA, and treatment scheduling information. Great effort was taken to establish the time from first symptom suggestive of CNV, such as when the patient first described metamorphopsia, new onset of central blur, or central scotoma. This information was collected by a retinal clinician to try to minimize incorrect interpretation of patient symptoms. Treatment delay from the time of the "first symptom" was measured in terms of weeks. Time from angiographic diagnosis to first treatment was also noted.

Best-corrected visual acuity (BCVA) was recorded for all patients using an Early Treatment of Diabetic Retinopathy Study (ETDRS) chart at each appointment.

All patients underwent a dilated fundus examination including fundus autofluorescence, with fluorescein angiography and optical coherence tomography (OCT) images being taken prior to enrollment in the study confirming their neovascular status. Angiograms were accessed via IMAGEnet 2000 for analysis (Topcon Corporation, Tokyo, Japan).

At each review appointment, BCVA, fundus examination, and imaging with OCT were repeated. All treatment decisions were based solely on the discretion of the treating retinal ophthalmologist. No specific retreatment strategies were implemented; however, 63% of patients received their first 3 injections at 4- to 6-week intervals and all consultants treated to obtain a retina without fluid on OCT before extending the interval between appointments.

Review of all angiograms was undertaken to classify lesion types and size. Lesion size was measured in comparison to optic disc area using the middle phase frame of the fluorescein angiogram. Color fundus photographs and angiographic images were reviewed to determine the presence of atrophy or fibrosis. Atrophy was defined as the presence of window defect in the early phase of angiogram that did not increase in size or intensity in the later phases. Fibrosis was documented if >25% of the neovascular complex showed staining rather than leakage beyond the borders of the lesion in the late phase.

The same data were gathered at each review appointment over a 6-month follow-up period. This period was

TABLE 1. Baseline Characteristics of Patients With Neovascular Age-Related Macular Degeneration, Characterized by Their Visual Outcome at a Period of 6 Months After Initiation of Treatment With Anti–Vascular Endothelial Growth Factor Therapy

Characteristics	Improved VA[a] (>2 line gain)	Stable VA[a] (<2 line gain or loss)	Decreased VA[a] (>2 line loss)	*P* Value
Age group (years)				
≤74	16 (42)	17 (45)	5 (13)	.27
75–84	28 (26)	67 (63)	12 (11)	
≥85	9 (23)	25 (63)	6 (14)	
Sex				
Male	27 (37)	31 (42)	15 (21)	.001
Female	26 (23)	78 (70)	8 (7)	
Smoking				
No smoking	19 (26)	46 (63)	8 (11)	.62
Past/current smoker	31 (31)	55 (56)	13 (13)	
Time delay: symptoms to treatment (weeks)				
Lowest tertile (<7)	21 (38)	28 (51)	6 (11)	.34
Middle tertile (7–21)	16 (30)	30 (56)	8 (14)	
Highest tertile (>21)	11 (20)	36 (67)	7 (13)	
Time delay: diagnosis of CNV to treatment (weeks)				
Lowest tertile (<1)	24 (29)	48 (57)	12 (14)	.42
Middle tertile (1–3)	17 (34)	26 (52)	7 (14)	
Highest tertile (>3)	11 (22)	33 (66)	6 (12)	
Baseline logMAR VA[b]				
Median (interquartile range)	0.70 (0.52)	0.60 (0.40)	0.48 (0.22)	.002
CNV lesion type				
Predominantly classic	13 (28)	27 (57)	7 (15)	.86
Non–predominantly classic[b]	39 (29)	81 (60)	16 (11)	
CNV lesion size				
≤2 DA	35 (31)	62 (55)	15 (14)	.37
> 2 DA	13 (21)	41 (67)	7 (12)	
Number of treatments				
≤3	23 (26)	54 (61)	11 (13)	.84
>3	28 (30)	54 (58)	11 (12)	

CNV = choroidal neovascularization; DA = disc areas; logMAR = logarithm of the minimal angle of resolution; VA = visual acuity.

[a]Data are shown as n (%).VA worsens as logMAR value increases.

[b]Includes minimally classic, occult, retinal angiomatous proliferation, fibrovascular pigment epithelial detachment.

selected based on data collected from the ANCHOR and MARINA studies[1–3] showing that most patients undergoing treatment declare whether they are going to respond or not respond in the first 3 to 6 months after commencement of treatment.

• STATISTICAL ANALYSIS: The primary outcome measurement of this case series was VA outcome following anti-VEGF treatment for neovascular AMD. To ensure sufficient numbers in the 3 defined outcome groups a change of 0.2 logMAR units was used. Improvement in visual acuity was defined as a decrease of 0.2 logMAR units in vision at 6 months after first treatment. Stable vision was defined as visual acuity within ±0.2 logMAR units of baseline after the same time period. Decreased acuity was considered to be an increase of 0.2 logMAR units or more at 6 months.

Individuals were recruited for subgroup analysis based on the following characteristics: 1) age (≤74, 75–84, ≥85 years); 2) sex (male or female); 3) smoking status (nonsmoker, past/current smoker); 4) treatment delay between first suggestive CNV symptoms and first treatment; 5) treatment delay between angiographically confirmed CNV and first treatment; 6) CNV lesion types (predominantly classic, non–predominantly classic); 7) lesion size (≤2 disc areas [DA], >2 DA); 8) injection number (≤3, >3); and 9) baseline VA. All of these treatment subgroups were selected before study commencement.

Data were reported as median and interquartile ranges (IQR) or mean ± standard deviations (SD) for continuous

variables, and as proportions for categorical variables. The χ^2 test was used to analyze categorical data. Differences in continuous variables among different treatment outcome groups were evaluated by Kruskal-Wallis rank test for skewed distributed data and analysis of variance (ANOVA) for normally distributed data. Ordinal logistic regression analyses were undertaken to investigate the association between these subgroups and treatment outcomes. The validity of the model assumption for parallel lines was essentially assessed. All potential explanatory variables (as either a continuous or discrete measure) were examined by univariate analysis to determine if they should be included in the final model. The complete and the reduced models, along with the logit link, were used to generate the candidate models. The complete model contained all the explanatory variables while the reduced model included a subset of the predetermined explanatory variables. All factors with a univariate significance level of $P < .10$, except for age and sex, were included in the multivariable ordinal. Finally, the best model was chosen among all candidate models based on the model fitting statistics, the accuracy of the classification results, the validity of the model assumption, and the principle of parsimony. Statistical analysis was performed using Stata Statistical Software Release 10.0 (Stata Corporation, College Station, Texas, USA). Odds ratios (OR) with 95% confidence intervals (CI) were determined to estimate the relative risk. A 2-tailed P value of <.05 was considered statistically significant.

RESULTS

• POPULATION CHARACTERISTICS: A total of 198 eyes from 192 patients were recruited into the study. Seven eyes of 7 patients failed to complete the follow-up period and were excluded. Of the 6 patients that had bilateral treatment, only the worse-seeing eye at 6 months was analyzed as we were particularly interested in determinants of poor outcomes. A total study population of 185 eyes of 185 patients was therefore analyzed for this study. Of these treated eyes, 75 had either bevacizumab or ranibizumab during the course of the treatment period and 110 had ranibizumab alone. The visual outcomes at 6 months were similar in the 2 groups, although a higher proportion of patients improved vision at 6 months in the ranibizumab group (n = 37, 34% vs n = 15, 20%). Counter to this, however, a larger number of eyes in the ranibizumab-only group lost vision at 6 months, n = 18/16% vs n = 5/6%. These differences were not statistically significant (P = .40) and hence the entire cohort was analyzed as a whole.

Table 1 summarizes the baseline characteristics of our study population. In this table the responses of the eyes treated with bevacizumab or ranibizumab were analyzed together and classified according to improvement in VA (n = 53, 29%), stable VA (n = 109, 59%), or decreased VA (n = 23, 12%).

TABLE 2. Baseline Clinical and Angiographic Features of Eyes With Neovascular Age-Related Macular Degeneration, Losing Vision at 6 Months, Following Initiation of Treatment With Anti–Vascular Endothelial Growth Factor Agents, Characterized by the Length of Delay From First Symptoms to Treatment Initiation

	Clinical/Angiographic Appearance*					
Delay to Presentation	Fibrosis	Atrophy	Chronic PED	Subretinal Hemorrhage	PC	NC
<7 weeks	2		1	2		1
7–21 weeks		2	2	1	2	1
>21 weeks	1	3	1		1	1

*Fibrosis: >25% staining of lesion on angiography; Atrophy: window defect in early phase of angiogram with loss of retinal pigment epithelium clinically; Chronic PED: chronic pigment epithelial detachment; Subretinal hemorrhage: large subfoveal hemorrhage with significant masking of details on angiography; PC: predominantly classic choroidal neovascular complex with no features of fibrosis or atrophy; NC: Non–predominantly classic choroidal neovascular complex with no features of fibrosis or atrophy.

The majority of study participants were in the 75–to–84 age group; 58% of patients were past or present smokers. The most common timing in treatment delay between the first symptoms suggestive of CNV (visual distortion, blur, scotoma) and first anti-VEGF injection was within the shortest tertile, that being less than 7 weeks (n = 55, 34%). Similarly, the shortest tertile of less than or equal to 1 week was the commonest delay between CNV diagnosis and first treatment (n = 84, 48.3%). Patients in this study population had mostly non–predominantly classic lesion types (n = 136, 74%), smaller than or equal to 2 DA (n = 112, 66%).

The percentage of study eyes that improved generally decreased as treatment delay from first symptoms was prolonged across the 3 VA categories of treatment response. Among those with a delay to treatment of 7 weeks or less, 38% of eyes improved vision at 6 months. This compared to improvement in only 20% of eyes where the delay was 21 weeks or more.

A similar percentage of eyes had a significant loss in vision during the study irrespective of the time delay between initial symptoms and treatment: 11% with <7 weeks delay, 14% with 7 to 21 weeks delay, and 13% with over 21 weeks. Looking specifically at the eyes that improved by 2 or more lines compared to those that did not, eyes treated within 7 weeks (n = 21, 38%) were much more likely to have improved vision compared to eyes treated >21 weeks (n = 11, 20%) after initial symptoms (χ^2 4.17, P = .04). Table 2 characterizes the characteristics

of these eyes with respect to baseline clinical and angiographic features.

Most eyes that lost vision had features of atrophy or fibrosis at baseline, with a smaller number having significant subretinal blood or chronic pigment epithelial detachment prior to treatment. No difference was noted in these characteristics with respect to extent of delay in presentation, although lesions with more atrophy appeared to be more prevalent in patients with longer delays in presentation and those with significant subfoveal hemorrhage tended to present earlier.

In eyes with good vision at baseline (<0.30 logMAR), we found a trend that eyes treated within 7 weeks of initial symptoms had a greater chance of maintaining or experiencing improvement in vision compared to eyes with delays of >21 weeks (90% vs 67%, $\chi^2 = 1.48$, $P = .48$). Where baseline vision was poor (logMAR >1.00), there was a significantly greater chance of vision improvement if treatment was prompt (80% vs 25%, $\chi^2 = 6.71$, $P = .05$).

Of the other baseline characteristics, men were more likely to improve with treatment than women but were also more likely to lose vision ($P = .001$). Baseline vision was associated with treatment outcome at 6 months. Those patients with better initial vision were more likely to deteriorate despite treatment, compared to those with poorer starting vision ($P = .002$). There was no correlation, however, between baseline vision and length of delay in presentation from first symptoms ($r = 0.14$, $P = .08$). Lesion size had no effect on treatment outcome ($P = .37$).

Considering the delay to presentation with regard to the visual acuity in the fellow eye, there were 102 eyes where the fellow eye had an acuity of <0.30 logMAR and 61 where the acuity was >0.30 logMAR. Of these 2 groups, the median delay to presentation was 12 weeks (range 0–107) in eyes with good vision in the fellow eye, compared to 10 weeks (range 0–89) in those eyes with impaired vision in the fellow eye ($P = .73$).

• UNIVARIATE ANALYSIS: The primary aim of univariate analysis was to assess whether any particular clinical characteristics impacted on VA outcomes with anti-VEGF treatment. As no covariate violated the parallel assumption of ordinal logistic regression analysis, we used this statistical methodology to compare the variables within each subgroup and their relationship with treatment outcomes.

The following characteristics were found to have a significant impact on "adverse" visual outcome (increased risk of deterioration in vision or reduced chance of visual improvement) at 6 months. Patients with a longer delay between their first visual symptoms of CNV (visual distortion, blur, scotoma) and their first anti-VEGF treatment were more likely to have poor treatment outcomes (OR 1.98; 95% CI 1.00, 4.08; $P = .05$. Better baseline VA was strongly associated with a greater loss of vision compared to those with poor vision at baseline (OR 0.30; 95% CI 0.15, 0.61; $P = .001$.

Age, sex, treatment delay from diagnosis of CNV (as seen on fluorescein angiography) to first treatment, lesion size, and lesion type had no influence on visual outcome ($P > .10$).

The finding of better baseline vision and longer delay to treatment from symptoms being associated with a greater chance of adverse outcome was also seen when eyes were characterized with respect to lesion type, delay to presentation, visual acuity in the fellow eye, and type of anti-VEGF agent used in treatment.

In terms of lesion type, we found that better baseline vision was a significant predictor of adverse vision outcome in both non–predominantly classic lesions (OR 0.23; 95% CI 0.08, 0.58; $P = .002$) and predominantly classic lesions (OR 0.47; 95% CI 0.20, 0.97; $P = .06$). Similarly, a delay of 21 or more weeks in presentation was seen to influence outcome in both lesion types, with the risk of adverse outcome being OR 2.1; 95% CI 0.97, 5.20; $P = .06$ in non–predominantly classic lesions and OR 2.06; 95% CI 0.93, 4.61; $P = .07$, in predominantly classic lesions.

To assess whether visual acuity in the fellow eye impacts on the finding that increasing delay to presentation affects visual outcome (as the estimation of delay might be more robust in the second eye if the first eye has poor vision), we analyzed eyes in 2 groups: those with "good" vision in the fellow eye (logMAR <0.30) and those with "bad" vision (logMAR >0.30).

Of the eyes with good vision in the fellow eye, we found that increasing age (OR 8.06; 95% CI 1.84, 35.30; $P = .006$), better baseline vision (OR 0.30; 95% CI 0.12, 0.76; $P = .01$), and increasing delay to presentation (OR 5.46; 95% CI 1.91, 15.7; $P = .002$) were likely to affect outcome adversely. In eyes with pre-existing bad vision in the fellow eye, similar trends were seen for increasing age (OR 1.62; 95% CI 0.30, 9.69; $P = .61$), sex (OR 0.37; 95% CI 0.10, 1.35; $P = .13$), increasing delay to presentation (OR 1.49; 95% CI 0.38, 5.87; $P = .56$), and better baseline vision (OR 0.20; 95% CI 0.02, 1.92; $P = .16$), although none were statistically significant.

With respect to the type of anti-VEGF agent used during treatment, we found that in both the combined bevacizumab and ranibizumab group and ranibizumab-alone group, baseline vision was a significant determinant of vision, $P = .004$ and $P = .02$ respectively, with better vision less likely to improve (or more likely to deteriorate). Delay of 21 weeks or more was associated with an increased likelihood of adverse visual outcome in both groups, although this was only significant in the ranibizumab-only group (OR 3.20; 95% CI 1.20, 8.50; $P = .02$), with a trend being seen in the combined group (OR 1.95; 95% CI 0.50, 8.50; $P = .30$).

We also considered the time delay between angiographic diagnosis of CNV and treatment. For this parameter we saw no significant difference in outcome when

TABLE 3. Multivariable Ordinal Logistic Regression Analysis Assessing the Influence of Baseline Patient Characteristics on Adverse Outcome (Vision Loss or Failure to Gain Vision) at 6 Months, Following Treatment Initiation With Anti–Vascular Endothelial Growth Factor Agents for Neovascular Age-Related Macular Degeneration

Characteristics	Odds Ratio for Adverse Treatment Outcome (95% CI)[a]	*P* Value[a]
Age (years)		
≤74	1	
75–84	2.07 (0.93, 4.64)	.07
≥85	2.88 (1.08, 7.70)	.04
Sex		
Male	1	.44
Female	0.77 (0.40, 1.48)	
Time delay: symptoms to treatment (weeks)		
Lowest tertile (<7)	1	
Middle tertile (7–21)	1.81 (0.83, 3.92)	.73
Highest tertile (>21)	2.62 (1.20, 5.68)	.015
Baseline logMAR VA[b]	0.25 (0.11, 0.57)	.001

CI = confidence interval; logMAR = logarithm of the minimal angle of resolution; OR = odds ratio; VA = visual acuity.

[a]Each OR and *P* value is adjusted for all other characteristics listed in the table.

[b]VA worsens as logMAR value increases.

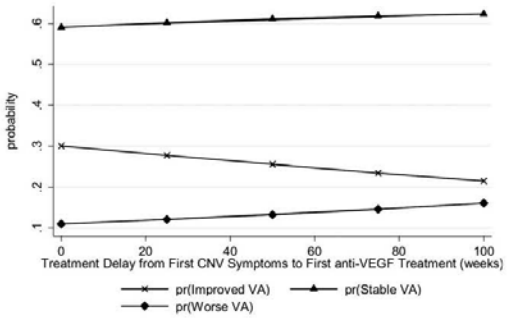


FIGURE. The predicted probability for change in vision at 6 months based on the timing of anti–vascular endothelial growth factor therapy initiation, from first symptoms of choroidal neovascularization, in eyes with age-related macular degeneration. pr, probability; VA, visual acuity; CNV, choroidal neovascularization; anti-VEGF, anti–vascular endothelial growth factor; Improved, ≥2 lines of improvement in acuity compared to baseline; Stable, within 2 lines of baseline; Worse, ≤2 lines of baseline visual acuity.

these shorter time periods were considered. In our study the time delay between angiographic diagnosis and treatment was uniformly short (median time for treatment from diagnosis was 2 weeks [interquartile range 1–4 weeks]), and when this delay was divided into <1 week, 1 to 3 weeks, and >3 weeks, no significant impact was seen with respect to adverse outcome (OR 0.82; 95% CI 0.40, 1.65; $P = .57$ for 1–3 weeks and OR 1.04; 95% CI 0.53, 2.06; $P = .90$ for >3 weeks delay).

• MULTIVARIATE ANALYSIS: All clinical characteristics with a significance level of $P < .10$ identified in the univariate analysis were incorporated into a multivariate analysis (Table 3). Age and sex was also included to be consistent with existing literature. Longer treatment delay from first symptoms of CNV was a highly significant predictor ($P = .015$) of adverse outcome when adjusting for age, sex, and baseline VA, with an OR of 2.62 (95% CI 1.20, 5.68). As such, a treatment delay of greater than 21 weeks, compared to 7 weeks or less, resulted in a 2.6-fold increase in the likelihood of adverse response despite treatment.

The Figure illustrates how the detailed predicted probabilities of the 3 treatment response groups changed over different lengths of delay.

• SHORTER DELAYS: A 7-week delay between initial symptoms and treatment reflects the time point where there was an even distribution of patients over the 3 time periods. We also looked at shorter time intervals to determine if this had any effect on outcome. Although not statistically significant ($P = .26$), patients presenting within 4 weeks of symptoms had a 38% (15/40) chance of a 2-line improvement in vision, compared to 33% improving with a 5- to 8-week delay and only 26% (25/102) in those delayed by more than 8 weeks. The likelihood of a poor response did not appear to be influenced by this delay, with 13% losing 2 or more lines of vision in the ≤4-week delay, 14% with 5- to 8-week delay, and 13% with >8-week delay. These difference were not statistically significant ($P = .67$).

DISCUSSION

THIS STUDY ANALYZED THE INFLUENCES UPON CLINIC-based treatment outcomes of patients receiving either bevacizumab or ranibizumab for neovascular AMD. Our analyses found that better baseline vision was more likely to be associated with adverse visual outcomes. This has been documented previously[9,10] and is likely explained by the "ceiling" effect, where it is hard to improve vision where the vision is already good at baseline. We also demonstrated that longer treatment delay from first symptoms suggestive of CNV to first anti-VEGF intravitreal injection is a significant predictor of adverse visual outcome ($P = .015$), independent of age, sex, and baseline VA. Our results suggest that shorter delays (<7 weeks) from initial symptoms to time of first treatment are more likely to result in improved vision than if the period is extended. Although not significant with delays of <4 weeks compared to delays of >8 weeks, the same trend was present. Thus, it appears that to gain vision, it is important to shorten the gap between first symptoms and initial treatment. Time delay was more influential in the chance of gaining vision than in determining those who lost vision. This observation cannot be simply explained by a correlation between presenting VA and delay, as this correlation was not significant ($r = 0.14$, $P = .08$). Additionally, baseline VA was included as a covariate in the multivariate analysis.

Presenting vision is affected by a number of factors, including, for example, degree of hemorrhage in the central macula, which would have a profound effect on presenting VA but may not have a long-term effect on VA, compared to factors such as intraretinal cysts, which may determine one's ability to improve VA. Thus delay in treatment may be more indicative of irreversible structural changes in the macula, which would influence the ability to improve vision. Duration of symptoms may therefore give a better prediction of the likelihood to improve than baseline vision. Delay to treatment was also found to be a significant influence on visual outcome in patients whose fellow eye had "good vision" at the time of diagnosis and, while a trend was noted in those eyes with "poor vision" in the fellow eye, who might be expected to be more accurate in identifying their first symptoms. Our findings suggest that even in patients with good vision in the fellow eye, where first symptoms of CNV may be less accurate than in those with a poor-seeing second eye, the time delay of symptoms to treatment still predicted the outcome.

Delays of 21 weeks or more led to a 5-fold increased risk of adverse visual outcome post-treatment, in comparison to a delay up to 7 weeks.

The MARINA[9] and ANCHOR[10] studies did not consider the delay to initiation of treatment from first CNV symptoms, assessing only the delay between CNV diagnosis and first treatment. They, like us, did not find any association between delay to initiate treatment after angiographic confirmation of CNV and outcome parameters. Given this lack of association between time from angiographic diagnosis of CNV to treatment and outcome, it would appear that a delay of 3 weeks does not adversely influence the outcome. This is important to know as there is often some inherent delay between diagnosis and treatment, especially where obtaining approval for the drug from relevant authorities can introduce a small delay.

We recognize that this assessment of time from symptoms is a less robust time interval than that from angiographic diagnosis to first treatment and is limited by the recall of patients being variable and also by an incorrect delay being recorded when symptoms are not recognized. However, we looked at the delay when people presented with either good vision in their other eye or bad vision in their other eye with the assumption that those with bad vision in the other eye might report more accurate symptom times. Our results did not differ between these 2 groups, suggesting that the vision in the fellow eye did not appear to influence reliability in determining symptom period.

The interval from first symptom to first treatment is a time where there is likely to be more variation between people, some with significant delays, than the delay after the confirmed angiographic diagnosis, where treatment is usually delivered as soon as possible. This important variable is not often accurately recorded in routine clinical notes, but it may well be one of the most important variables to consider as we try to determine the predictors of outcome after anti-VEGF treatments. Also, this time interval is one that could be improved compared to other parameters, such as genetic predisposition, which cannot be altered. Despite this, no other study that we know of has attempted to obtain this information prospectively, and the majority of patients, upon detailed questioning, could give an answer as to the duration of symptoms.

There were some individuals diagnosed with stable occult lesions where the decision to treat was delayed until evidence of progression was seen. This suggests that the onset of symptoms may have been more gradual, not provoking immediate treatment. However, of the patients with chronic occult lesions that were left untreated until evidence of progression was documented, 50% improved vision by 2 or more lines with treatment, 33% were stable, and 17% deteriorated. This suggests that the delay introduced in treating these individuals did not affect their ability to respond positively to treatment. It is unclear, however, whether they would have had a superior response had they been treated earlier in the course of disease.

As for the underlying reasons for the suboptimal visual outcome following treatment in those with longer delays, it is possible that the longer the presence of subretinal and/or intraretinal fluid associated with choroidal neovascularization, the greater the likelihood of permanent damage to

the photoreceptor/retinal pigment epithelium complex. Thus, prompt treatment may allow less disruption of macular anatomy and increasing delay causes irreversible changes that prevent the vision from improving rather than being responsible for the vision's continued decline. This fits very well with our findings that the time delay was more influential in the chance of gaining vision than in determining those who lost vision.

The strengths of our study include a prospective design and a study population drawn directly from the clinic, allowing accurate representation of normal clinic patients and normal treatment procedures. We also set out to specifically ask a question about the delay from first symptom suggestive of CNV to first treatment. A potential limitation is that there is no definitive way of verifying the time delay and the patients' interpretation of their visual symptoms. Secondly, the study traversed a period where both ranibizumab and bevacizumab were used to treat patients with neovascular AMD, and most patients had a combination of these treatments at various points in the study. Although both drugs have similar mechanisms of action and several studies have found no significant differences in outcome following treatment,[8,13,14] we cannot be absolutely sure whether the specific medication given could have influenced outcome. Finally, there are factors that may account for poor visual outcome independent of those that we considered in this study. Such factors as pigment epithelial tears during the course of treatment or significant subretinal hemorrhage prior to treatment may compromise outcome, despite prompt presentation and timely treatment.

It is of great interest that there were exceptions to the rule: even despite a short delay some patients did badly, and a long delay did not always mean that vision could not improve. There were 11 people in the longest-delay group who still improved vision by 2 lines despite this very long delay and 6 people in the shortest-delay period who lost 2 lines of vision despite a short delay (Table 1). Thus, there may well be other important factors influencing outcome, such as treatment protocols and baseline features such as atrophy and fibrosis, which may be partly determined by extent of delay or individual genetic make-up.

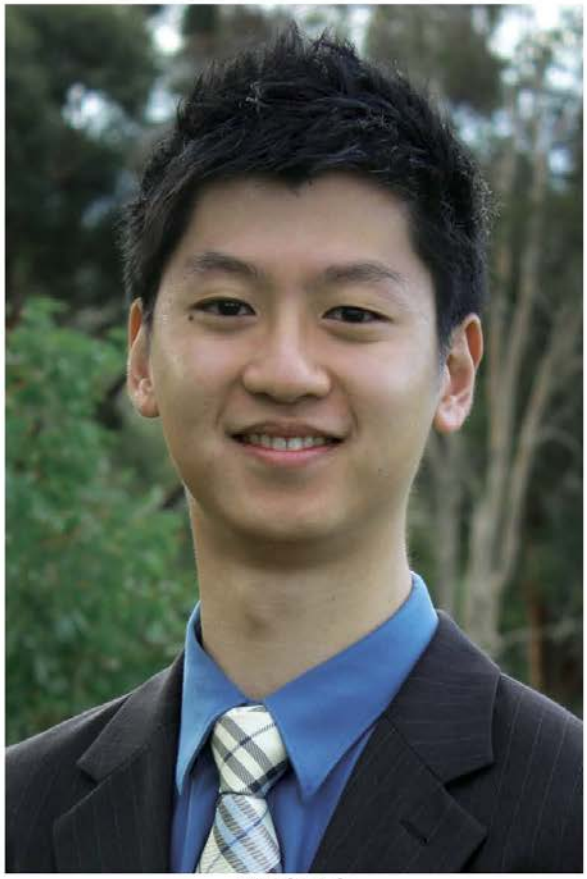
In summary, we demonstrate a significant association between longer anti-VEGF treatment delay from first CNV symptoms and poor VA outcome, particularly in the ability to gain vision with treatment. Our findings suggest that, at least in active lesions, it is imperative to act quickly to minimize treatment delay following initial symptoms suggestive of CNV. Our findings reiterate the dogma that any visual symptom of distortion, blur, or central scotoma should be regarded as needing an urgent referral for evaluation, if we are to maximize the benefit of anti-VEGF therapies.

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Biosketch

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Biosketch

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Predictors of AMD Treatment Response

Dear Editor:

It is important that we understand which factors dictate the visual acuity outcome in people receiving anti-vascular endothelial growth factor (anti-VEGF) for choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD).[1–3] In this study, we sought to assess whether optical coherence tomography (OCT) features at baseline and after initial treatment correlated with visual outcome at 12 months. This study was approved by the Human Research and Ethics Committee of the Royal Victorian Eye and Ear Hospital.

Treatment-naïve patients diagnosed with subfoveal CNV secondary to AMD were prospectively consecutively recruited from the Medical Retina Clinic at the Royal Victorian Eye and Ear Hospital and the private rooms of the Eastern Retinal Service in Box Hill, Melbourne, Australia. All patients received 3, monthly injections of ranibizumab and/or bevacizumab, with subsequent retreatment decisions at monthly visits based on a modification of the SUSTAIN study.[4] In brief, retreatment was performed if there was either persistence or recurrence of intraretinal fluid or subretinal fluid on OCT, loss of ≥1 lines of vision, or the presence of new macular hemorrhage on clinical examination.

At baseline, OCT was assessed for the presence of an intraretinal fluid (IRF) component (cysts or edema), subretinal fluid (SRF), pigment epithelial detachment (PED), and a significant band of reflectance at the retinal pigment epithelial (RPE) level, because it has been suggested that this is a sign of irreversible damage to the outer retina/RPE.[5] After 3 injections, eyes without IRF or SRF were considered "dry," as were eyes with PED without IRF or SRF. Regardless of the presence of PED, if either IRF or SRF were present, eyes were considered as having "persistent fluid" (Fig 1A, B, available at http://aaojournal.org). The presence or absence of a hyperreflective RPE band was considered separately as it could be present irrespective of the nature of the fluid status (Fig 1C, D).

At baseline, 144 of 214 eyes (67.2%) had IRF with or without SRF and 70 (32.8%) eyes had SRF alone. Of 58 eyes (27.1%) that had PED, 38 had associated IRF, and 20 had additional SRF alone (Table 1, available at http://aaojournal.org). Best-corrected logMAR visual acuity (BCVA) at baseline was significantly worse in eyes with IRF (0.79 [Snellen equivalent, 20/122]; 95% confidence interval [CI], 0.68–0.90) compared with eyes with SRF alone (0.55 [20/72]; 95% CI, 0.34–0.75; $P = 0.006$). The presence of PED did not significantly impact on BCVA. Data at the 12-month visit were available for 187 eyes (87.4% of recruited eyes). No differences were seen with respect to the baseline population and the 187 eyes with complete data available at 12 months ($P>0.10$). Over 12 months, 26.2% of eyes gained ≥2 lines of BCVA compared with 19.3% ≥3 line gain in the SUSTAIN study.4 51.3% remained stable (±2 lines) and 22.5% lost ≥2 lines. The location of fluid at baseline did not significantly influence the likelihood of BCVA improvement at 12 months, although eyes with SRF at baseline had better BCVA (0.54 [20/69]; 95% CI, 0.34–0.75) compared with those with an IRF component (0.78 [20/120]; 95% CI, 0.63–0.93; $P = 0.07$). After the 3 initial injections, 63.1% of eyes were classified as being dry, whereas 26.2% had IRF and 10.7% had residual SRF only. The factors influencing BCVA change at 12 months are shown in Table 2 (available at http://aaojournal.org). After 3 injections, eyes that were dry had better BCVA at 12 months (0.61 [20/80]; 95% CI, 0.52–0.70) compared with those with residual IRF (0.95 [20/180]; 95% CI, 0.78–1.11; $P = 0.05$). Eyes with SRF alone (0.65 [20/90]; 95% CI, 0.39–0.91) were similar to those that were dry. The presence of persistent fluid was associated with a reduced chance of visual improvement at 12 months (18.6% vs 31.4%; $P = 0.04$).

At baseline, 6 eyes demonstrated RPE hyperreflectance. This increased to 29 (15.5%) after 3 injections. Regardless of the presence or absence of fluid, hyperreflectivity conferred a greater likelihood of BCVA loss (48.3% vs 19.7%; $P = .006$) and poorer final BCVA (0.89 [20/155]; 95% CI, 0.69–1.10) compared with eyes without hyperreflectance (0.65 [20/90]; 95% CI, 0.56–0.73; $P = 0.02$). Two thirds (66%) of eyes with PED at baseline were noted to have residual PED after initial injections with no difference in final BCVA dependent on the PED.

After 3 treatments, eyes with a residual IRF component were associated with a greater chance of BCVA loss at 12 months compared with those who were dry (odds ratio [OR], 6.08; 95% CI, 1.53–32.56; $P = 0.01$), whereas those with RPE hyperreflectivity were also more likely to lose BCVA (OR, 4.48; 95% CI, 1.11–16.41; $P = 0.03$; Tables 3 and 4, available at http://aaojournal.org). Our findings suggest that the presence of IRF fluid at baseline leads to worse presenting acuity and confers a significantly worse prognosis for visual outcome.

Failure to clear fluid from the retina after the loading dose injections also correlates with poorer BCVA outcome and with a greater chance of losing vision at month 12 (Fig 2A, B, available at http://aaojournal.org). This may relate to disruption of retinal architecture with IRF as opposed to SRF, where the retina may be relatively intact.

Failure to resolve fluid from the retina after initial treatment may be a marker of irreversible structural damage to the photoreceptor/RPE complex. Most eyes with persistent fluid after 3 injections also had persistent fluid at 12 months, suggesting that the ability to dry the retina is determined early in the course of treatment. Early in the course of treatment, RPE hyperreflectance also correlates with BCVA loss at 12 months. Most eyes with this feature had significant subretinal hemorrhage at baseline. Of the initial 214 eyes, 29 had significant hyperreflectance at the RPE level. Of these, some degree of atrophy or subretinal fibrosis was identified in 21 eyes, and of the remaining 8 eyes, 7 had residual subretinal hemorrhage, which by 12 months revealed atrophy (n = 2) or subretinal fibrosis (n = 5).

Table 5 (available at http://aaojournal.org) details findings of eyes with RPE hyperreflectance. No association was seen with presence of PED at baseline or after 3 treatments and BCVA change at 12 months. Anti-VEGF treatment however, did reduce the frequency of PED compared with baseline.

In conclusion, eyes with IRF after initial treatment may require more aggressive treatment strategies to achieve best possible outcome, whereas a significant RPE hyperreflective band limits the potential for visual improvement.

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Voriconazole for *Candida* Endophthalmitis

Dear Editor:

Endogenous fungal endophthalmitis is a serious sight-threatening complication of systemic fungemia. Infectious Diseases Society of America (IDSA) guidelines for the management of endogenous *Candida* endophthalmitis recommend intravenous amphotericin-B and oral flucytosine, possibly with vitrectomy.[1,2] Recently, there have been reports of using intravitreal voriconazole injection as a treatment option in fungal endophthalmitis, including *Aspergillus*, *Fusarium*, *Scedosporium, and Candida*. To the best of our knowledge, the following is the first reported case of combined multiple voriconazole injection and systemic medication in a patient with bilateral endogenous *Candida* endophthalmitis. We searched PubMed, MEDLINE, and Google Scholar in March 2012 for key words including bilateral endogenous fungal endophthalmitis, *Candida* endophthalmitis, drug-resistant fungal endophthalmitis, and multiple intravitreal voriconazole injection (all languages without dates restriction), which showed no prior reports of bilateral *Candida* endophthalmitis successfully treated with multiple intravitreal voriconazole injections combined with systemic medication.

A 69-year-old man with diabetic mellitus, was referred to our emergency room due to pain and blurring vision in both eyes for 2 weeks. He had undergone right percutaneous nephrolithotomy 1 month ago at another hospital, complicated with subsequent *Candida* urosepsis. Urine culture showed *Candida albicans*. Then days later, he undergone cholecystectomy due to cholecystitis, but further blood culture revealed *Candida albicans*. Following this, intravenous fluconazole was prescribed for 9 days. Fundus examination revealed multifocal yellow-white lesions with fluffy borders, severe vitritis with "string-of-pearls" signs in both eyes, compatible with the diagnosis of *Candida* endophthalmitis (Fig 1A–B; available at http://aaojournal.org). Visual acuity was counting fingers only in both eyes. There was anterior chamber flare++ and cells++. Optical coherence tomography (OCT) showed multiple chorioretinal lesions with marked retinal thickening, protrusion, and poor macular contour (Fig 2A–B; available at http://aaojournal.org).

In addition to systemic therapy with amphotericin-B and flucytosine, the patient was treated with intravitreal voriconazole injection (100 μg/0.1 ml) immediately, again at the time of vitrectomy in both eyes 3 days later after systemic condition stabilized, and further weekly intravitreal injection. After 6 weeks of treatment, retinal lesions showed regression, and visual acuity improved from counting fingers to 1/60 in right eye and 6/60 in left eye. Fundus examination (Fig 3A–B available at http://aaojournal.org) and OCT (Fig 4A–B; available at http://aaojournal.org) 4 months after treatment demonstrated significant improvement of the endophthalmitis with resolution of *candida* chorioretinitis and subretinal chorioretinal scarring. Later cultures of blood, urine, and vitreous were negative for fungus growth. Latest ocular examination reported stable visual acuity.

Voriconazole, a promising broad-spectrum triazole agent, solves difficult cases as failure in first-line treatment or drug-resistant fungus. For vision-threatening macular involvement, intravitreal voriconazole injection is an effective way for rapid achievement of therapeutic concentration with minimal systemic side effect. Instead of single injection, the current case was treated with multiple injections during the treatment course. In a study regarding the clearance profile of intravitreal voriconazole, injecting 35 μg/0.1 mL into vitreous cavity of rabbit yielded a fast decline of concentration, with half-life of 2.5 hours and rapid decay within 24 hours after injection.[3] The authors suggest that supplementation of intraocular voriconazole to maintain therapeutic levels might be required.[3] An in vitro safety study claimed that concentrations <250 μg/mL of voriconazole had no influence neither on human retinal pigmented epithelium nor on optic nerve head astrocytes cell proliferation and cell viability.[4] In our case, the estimated concentration after injecting voriconazole 100 μg/0.1 ml into vitreous cavity (which became 2.5 μg/ml in a 4 ml-volume vitreous cavity) doesn't exceed the safety range.

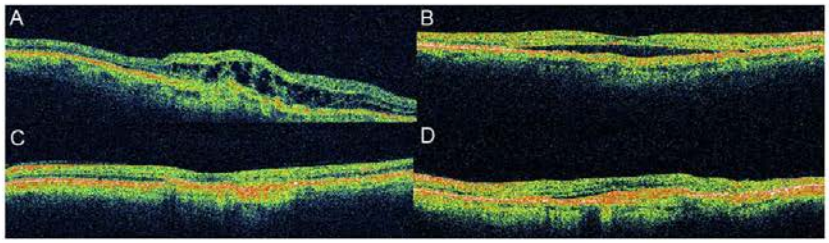


Figure 1. A, Optical coherence tomography scans depicting intraretinal fluid (IRF), (B) subretinal fluid (SRF), and a hyperreflective retinal pigment epithelial (RPE) band, with (C) or without (D) fluid.

Table 1. Background and Treatment Characteristics of 214 Eyes Included in the Study

Characteristic	n	%
Age group (yrs)		
<75	43	20.1
75–84	124	57.9
≥85	47	22.0
Gender		
Male	83	38.8
Female	131	61.2
Smoking status, if known		
Never smoker	81	42.2
Smoker (past or present)	111	57.8
Type of CNV lesion		
Predominantly classic	54	25.2
Nonpredominantly classic*	160	74.8
Size of CNV (DA)		
≥2	136	63.5
>2	78	36.5
Baseline OCT appearance		
IRF with SRF	144	67.2
SRF	70	32.8
Hyperreflectivity of RPE	6	2.8
Treatment type		
Ranibizumab and bevacizumab combination	72	33.6
Ranibizumab	142	66.4
Delay in seeking treatment, median weeks (IQR)	12.0 (5.0–26.0)	
Baseline BCVA, median (IQR)	0.60 (0.42–0.86)	
No. of injections at 12 months, median (IQR)	5 (3–7)	

BCVA = best-corrected logMAR visual acuity; CNV = choroidal neovascularization; DA = disc areas; IQR = interquartile range; IRF = intraretinal fluid; OCT = optical coherence tomography; RPE = retinal pigment epithelium; SRF = subretinal fluid.
*Includes minimally classic, occult, retinal angiomatous proliferation, fibrovascular RPE detachment.

Table 2. Visual Outcome at 12 Months Based on Baseline and Treatment Characteristics of Study Eyes

Characteristic	Change in BCVA at 12 Months			P Value
	Improved BCVA (>2 Line Gain; n = 49; 26.2%)	*Stable BCVA (≤2 Line Gain or Loss; n = 96; 51.3%)*	*Decreased BCVA (>2 Line Loss; n = 42; 22.5%)*	
Age group, n (%), yrs				0.37†
<75	14 (35.9)	18 (46.2)	7 (17.9)	
75–84	26 (24.5)	58 (54.7)	22 (20.8)	
≥85	9 (21.4)	20 (47.6)	13 (31.0)	
Gender, n (%)				0.01†
Male	23 (31.9)	27 (37.5)	22 (30.6)	
Female	26 (22.6)	69 (60.0)	20 (17.4)	
Smoking status if known, n (%)				0.38†
Never smoker	22 (31.9	34 (49.3)	13 (18.8)	
Smoker (past or present)	23 (23.0)	52 (52.0)	25 (25.0)	
Type of CNV lesion, n (%)				0.13†
Predominantly classic	18 (36.7)	20 (51.3)	11 (22.4)	
Nonpredominantly classic*	31 (22.5)	76 (55.1)	31 (22.5)	
Size of CNV, n (%), DA				0.44†
≤2	34 (29.0)	59 (50.4)	24 (20.5)	
>2	16 (22.8)	35 (50.0)	19 (27.2)	
Delay in seeking treatment (weeks), median (IQR)	11.5 (4.0–31.0)	12.0 (4.5–26.0)	12.0 (6.5–21.5)	0.06‡
Baseline BCVA, median (IQR)	0.69 (0.48–1.00)	0.60 (0.33–0.78)	0.60 (0.48–0.78)	0.22‡
OCT appearance at baseline, n (%)				
IRF with or without SRF	42 (29.2)	66 (45.8)	36 (25.0)	0.49†
SRF	22 (31.4)	38 (54.2)	10 (14.2)	
Hyperreflectivity of RPE	1 (17.0)	3 (50.0)	2 (33.0)	0.54†
Pigment epithelial detachment	11 (19.0)	27 (47.6)	20 (32.4)	0.26†
Treatment type				
Ranibizumab/bevacizumab combination	16 (23.5)	36 (52.9)	16 (23.5)	0.74†
Ranibizumab	34 (28.6)	60 (50.4)	25 (21.0)	
No. of injections at 12 months, median (IQR)	6 (4–8)	5 (4–7)	4 (3–5)	0.01‡
Residual fluid on OCT at 3 months, n (%)				
No residual fluid/dry	37 (31.4)	59 (50.0)	22 (18.6)	
IRF with or without SRF	9 (18.4)	22 (44.9)	18 (36.7)	0.15†
SRF	4 (20.0)	9 (45.0)	7 (35.0)	
Pigment epithelial detachment on OCT at 3 months, n (%)				
Yes	10 (25.8)	17 (45.2)	11 (29.0)	0.85†
No	40 (26.7)	73 (49.2)	36 (24.2)	
RPE hyperreflectance on OCT at 3 months, n (%)				
Hyperreflectivity of RPE	5 (17.2)	10 (34.5)	14 (48.3)	
No hyperreflectivity	45 (28.7)	82 (51.6)	31 (19.7)	0.006†

BCVA = best-corrected LogMAR visual acuity; CNV = choroidal neovascularization; DA = disc areas; OCT = optical coherence tomography; IQR = interquartile range; IRF = intraretinal fluid; RPE = retinal pigment epithelium; SRF = subretinal fluid.
*Includes minimally classic, occult, retinal angiomatous proliferation, fibrovascular RPE detachment.
†Chi square test.
‡Analysis of variance test.

Table 3. Results of Generalized Estimating Equations Modeling of the Effects of Patient and Treatment Characteristics on Visual Acuity Outcomes at 12 Months

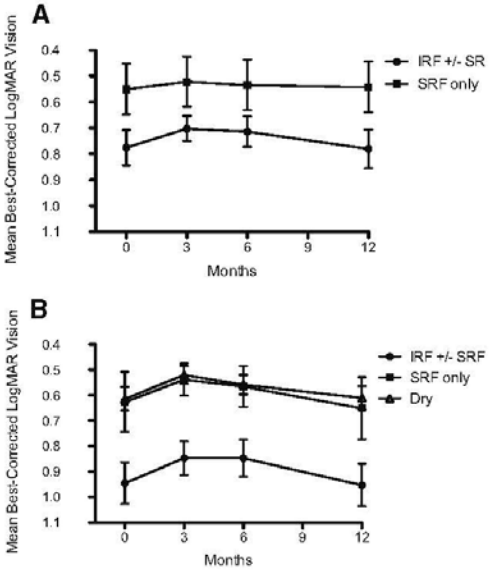
Predictor	Improved vs Stable BCVA*			Improved vs Decreased BCVA*		
	OR	95% CI	P Value	OR	95% CI	P Value
OCT appearance						
No residual fluid (dry)	1.00	Reference		1.00	Reference	
IRF with or without SRF	1.93	0.56–6.30	0.27	6.08	1.53–24.14	0.01
SRF	1.62	0.36–7.30	0.53	4.31	0.40–46.92	0.23
Male gender	1.86	0.72–4.80	0.20	0.52	0.16–1.72	0.28
No. of injections	0.87	0.70–1.07	0.19	0.67	0.50–0.88	0.004
Delay in treatment	0.98	0.96–1.00	0.15	0.97	0.95–1.02	0.06

BCVA = best-corrected LogMAR visual acuity; CI = confidence interval; IRF = intraretinal fluid; OCT = optical coherence tomography; OR = odds ratio; SRF = subretinal fluid.
*Reference group is improved VA.

Table 4. Results of Generalized Estimating Equations Modeling of the Effects of Patient and Treatment Characteristics on Visual Acuity Outcome at 12 Months

Predictor	Improved vs. Stable BCVA*			Improved vs. Decreased BCVA*		
	OR	95% CI	P-Value	OR	95% CI	P-Value
OCT appearance						
No hyperreflectivity of RPE	1.00	Reference		1.00	Reference	
Hyperreflectivity of RPE	1.32	0.26–6.66	0.74	4.48	1.11–16.41	0.03
Male gender	1.60	0.62–4.13	0.33	0.44	0.14–1.39	0.16
Number of injections	0.85	0.69–1.04	0.12	0.74	0.56–0.98	0.03
Delay in treatment	0.99	0.96–1.01	0.20	0.97	0.95–1.02	0.06

BCVA = best-corrected logMAR visual acuity; OR = odds ratio; CI = confidence interval; SRF = subretinal fluid; IRF = intraretinal fluid.
*Reference group is improved VA.



IRF, intraretinal fluid; SRF, subretinal fluid

Error bars represent the standard error of the mean LogMAR visual acuity

Figure 2. A, Change in mean visual acuity over time, depending on location of fluid within the macula on optical coherence tomography. B, Change in mean visual acuity over time, depending on presence and location of fluid within the macula on optical coherence tomography after 3 antivascular growth factor treatments.

Table 5. Clinical Features of Patients with a Band of Hyperreflectivity at the Level of the Retinal Pigment Epithelium (RPE) on Optical Coherence Tomography (OCT) after 3 Antivascular Endothelial Growth Factor Treatments

	Clinical Appearance			
OCT Appearances	*Fibrosis*	*Atrophy*	*Chronic PED*	*Subretinal Hemorrhage*
Hyperreflectivity with residual fluid (n = 14)	8	1	0	5
Hyperreflectivity with no residual fluid (n = 15)	4	8	1	2

atrophy = window defect in early phase of angiogram with loss of retinal pigment epithelium clinically; fibrosis = >25% of lesion is fibrotic scar; PED = retinal pigment epithelial detachment; subretinal hemorrhage = large subfoveal hemorrhage with significant masking of details on angiography.

Genetic Influences on the Outcome of Anti-Vascular Endothelial Growth Factor Treatment in Neovascular Age-related Macular Degeneration

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Purpose: To determine the association of genetic variants in known age-related macular degeneration (AMD) risk-associated genes with outcome of anti-vascular endothelial growth factor (VEGF) treatment in neovascular AMD.

Design: Prospective cohort study.

Participants: We enrolled 224 consecutive patients with neovascular AMD at the Royal Victorian Eye and Ear Hospital, Australia.

Methods: Patients were treated with 3 initial monthly ranibizumab or bevacizumab injections followed by 9 months of "as required" injections based on clinician's decision at each follow-up visit according to retreatment criteria. Seventeen single nucleotide polymorphisms (SNPs) in known AMD risk-associated genes including *CFH* (rs800292, rs3766404, rs1061170, rs2274700 and rs393955), *HTRA1* (rs11200638), *CFHR1-5* (rs10922153, rs16840639, rs6667243, and rs1853883), *LOC387715/ARMS2* (rs3793917 and rs10490924), *C3* (rs2230199 and rs1047286), *C2* (rs547154), *CFB* (rs641153) and *F13B* (rs6003) were examined. Multivariate analysis was used to determine the role of each SNP in treatment outcome.

Main Outcome Measures: The influence of selected SNPs on mean change in visual acuity (VA) at 12 months.

Results: Mean baseline VA was 51±16.8 Early Treatment Diabetic Retinopathy Study letters. Overall, the mean change in VA from baseline was +3.2±14.9 letters at 12 months. The AA (homozygote risk) genotype at rs11200638 - *HTRA1* promoter SNP ($P = 0.001$) and GG (homozygote risk) genotype at rs10490924 (*A69S*) in *LOC387715/ARMS2* ($P = 0.002$) were each significantly associated with poorer VA outcome at 12 months after multiple correction. Mean ± standard deviation change in VA from baseline in patients with AA genotype at rs11200638 was −2.9±15.2 letters after 12 months compared with +5.1±14.1 letters in patients with AG or GG genotypes at this SNP. Patients with either of these genotypes were also significantly more likely to lose >15 letters after 12 months. SNPs rs11200638 and rs10490924 were in high linkage disequilibrium ($r^2 = 0.92$). None of the other examined SNPs was associated with outcome.

Conclusions: The *HTRA1* promoter SNP (rs11200638) and *A69S* at *LOC387715/ARMS2* were associated with a poorer visual outcome for ranibizumab or bevacizumab treatment in neovascular AMD, suggesting strong pharmacogenetic associations with anti-VEGF treatment. This finding could aid in applying more individualized treatment regimens based on patients' genotype to achieve optimal treatment response in AMD.

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Intravitreal anti-vascular endothelial factor (VEGF) treatment with ranibizumab or bevacizumab has revolutionized the treatment of neovascular age-related macular degeneration (AMD).[1-3] However, the outcome of this novel treatment is not uniform in all patients. The results of the international pivotal studies, ANCHOR (*AN*ti-VEGF Antibody for the Treatment of Predominantly Classic *CHOR*oidal Neovascularization in Age-related Macular Degeneration) and MARINA (*M*inimally Classic/Occult Trial of the Anti-VEGF Antibody *R*anibizumab *I*n the Treatment of *N*eovascular Age-related Macular Degeneration), showed that whereas 30% to 40% of patients gained >15 Early Treatment Diabetic Retinopathy Study (ETDRS) letters, 5% to 10% lost >15 letters and the rest maintained visual acuity (VA) with monthly ranibizumab injections.[1,2] More recently, the Comparison of Age-Related Macular Degeneration Treatments Trials (CATT) and Inhibit VEGF in Age-related Choroidal Neovascularisation (IVAN) study groups reported similar effects for ranibizumab and bevacizumab on best-corrected visual acuity in patients with neovascular AMD.[3,4]

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Multiple studies have now reported single nucleotide polymorphisms (SNPs) in the complement factor H (*CFH*) gene,[5-8] Age-Related Maculopathy Susceptibility 2 (*LOC387715/ARMS2*), and high temperature requirement factor A1 (*HTRA1*) genes,[9-18] complement factor 2 (*C2*) gene, complement factor 3 (*C3*) gene, and the complement factor B (*CFB*) gene[19] are associated with higher risk of AMD.

Because anti-VEGF treatment outcome in patients with neovascular AMD is not uniform, research has focused on the association of genetic variants with different response patterns. Previously, some association had been reported between specific genotypes and response to photodynamic therapy in neovascular AMD.[20-22] With regard to anti-VEGF treatment outcome, McKibbin et al[23] identified trends for better VA outcome in patients with AMD risk genotypes at *VEGF*, *CFH*, and *HTRA1* genes after 6 months intravitreal ranibizumab treatment. Teper et al[24] indicated that patients homozygous for the T risk allele at *A69S* of the *ARMS2* gene had a poor best-corrected VA outcome with ranibizumab treatment. In a prospective study by Nischler et al,[25] individuals with the CC risk genotype of *Y402H* in the *CFH* gene were associated with a significantly worse VA outcome for bevacizumab treatment in neovascular AMD. This was reproduced in another study where patients with TT or TC genotypes at this SNP showed a better response to ranibizumab treatment compared with those with the homozygote risk CC genotype.[26] We have also previously reported that patients with apolipoprotein E (*APOE*) ε4 AMD protective allele showed a significantly better VA outcome with anti-VEGF treatment compared with those who do not carry this allele.[27] The CATT study group recently reported that there was no association between SNPs rs1061170 (*CFH*), rs10490924 (*ARMS2*), rs11200638 (*HTRA1*), and rs2230199 (*C3*) and the outcome of ranibizumab or bevacizumab treatment in neovascular AMD (Hagstrom SA. Pharmacogenetics of Anti-VEGF Therapy in the Comparison of AMD Treatments Trials [CATT]. Paper presented at: ARVO Annual Meeting, May 8, 2012, Florida). Although the results of these studies were not consistent, they suggest that a pharmacogenetic association is likely to play some role in the outcome of intravitreal ranibizumab or bevacizumab in patients with neovascular AMD.

In this prospective study, we examined the contribution of 17 SNPs in known AMD risk-associated genes, including *CFH* (rs800292, rs3766404, rs1061170, rs2274700, and rs393955), *HTRA1* (rs11200638), *CFHR1-5* (rs10922153, rs16840639, rs6667243, and rs1853883), *LOC387715/ARMS2* (rs3793917 and rs10490924), *C3* (rs2230199 and rs1047286), *C2* (rs547154), *CFB* (rs641153), and *F13B* (rs6003) to the outcome of anti-VEGF treatment in treatment-naïve patients with neovascular AMD.

Methods

Study Design and Eligibility

We prospectively recruited patients from the retina clinics of The Royal Victorian Eye and Ear Hospital (RVEEH) from 2006 to 2010 for this study. Approval was obtained from the human research and ethics committee of the RVEEH. Research adhered to the tenets of the Declaration of Helsinki, and all patients provided written informed consent before participation.

Patient Selection

Our study cohort comprised consecutive, treatment-naïve patients >50 years of age with active subfoveal choroidal neovascularization (CNV) secondary to AMD who commenced intravitreal ranibizumab or bevacizumab treatment in the RVEEH retina clinics. Patients were identified by retinal specialists, experienced in the management of AMD, on the basis of retinal examination, fundus photography, fundus fluorescein angiography (FFA), and time-domain optical coherence tomography (OCT) with Stratus OCT version 5.0.1 (Carl Zeiss Meditec, Dublin, CA) or Cirrus HD-OCT version 6.0.0.599 (Carl Zeiss Meditec). The presence of subfoveal leakage on FFA was required to make the diagnosis of active subfoveal CNV. All patients were followed for 12 months from their first injection. If both eyes of a patient were eligible at baseline, only 1 eye was included on a random basis. The fellow eye was treated at the treating clinician's discretion but not included in this study. During the course of the study, if the fellow eye developed CNV, this was again treated at the discretion of the treating clinicians but not included in the study.

Patients with CNV secondary to non-AMD conditions such as angioid streaks, severe myopia, central serous retinopathy, or hereditary retinal disorders and those who received any previous treatment for neovascular AMD such as anti-VEGF treatment, photodynamic therapy, or laser photocoagulation were not included. We excluded the patients who developed any serious adverse events over the course of the study as well as those with non-Caucasian ancestry.

Data Collection and Treatment

Patient demographics and lesion characteristics were recorded and a peripheral blood sample was collected for DNA extraction at the baseline visit. Lesion type and CNV size in comparison to optic disc area were determined by an experienced medical retina specialist (S.W.) using the middle phase frame of FFA at baseline. At baseline and each follow-up visit, patients underwent a dilated fundus examination, OCT imaging, and VA assessment. Qualified examiners assessed VA using the ETDRS charts at 4 meters.

The treatment included intravitreal injections of 0.5 mg ranibizumab (Lucentis; Novartis Pharma AG, Basel, Switzerland) or 1.25 mg bevacizumab (Avastin; Roche, Basel, Switzerland). Most patients received only ranibizumab throughout the duration of the study, although 21% received a single dose of bevacizumab at initial presentation while awaiting government approval for ranibizumab.

All patients received 3 initial monthly injections followed by a flexible as needed *pro re nata* (PRN) regimen until month 12. In the PRN phase, the treatment decision at each visit was at the discretion of the treating retinal specialist, based on the retreatment criteria including a loss of ≥5 letters in VA, evidence of persistence or recurrence of intraretinal or subretinal fluid on OCT, or presence of new or persistent hemorrhage on fundus examination. After 3 initial monthly injections, the subsequent follow-up visits were scheduled at monthly intervals. An extension by 2 weeks was considered for the subsequent follow-up visit if the patient was clinically stable and none of the retreatment criteria were present. The main outcome measure was association of variants in any of the 17 examined SNPs with mean change in VA after 12 months of treatment.

Genotyping

A peripheral blood sample was obtained from each patient and a total of 17 SNPs in known AMD-associated genes, including SNPs rs800292, rs3766404, rs1061170, rs2274700, and rs393955 in the *CFH* gene, SNP rs11200638 in *HTRA1,* SNPs rs10922153, rs16840639, rs6667243, and rs1853883 in *CFHR1-5* genes, SNPs rs3793917 and rs10490924 (*A69S*) in *LOC387715/ARMS2*, SNPs rs2230199 and rs1047286 in *C3*, SNP rs547154 in *C2*, SNP rs641153 in *CFB* and SNP rs6003 in *F13B,* were genotyped.

Genotyping was performed on the MassArray platform (SEQUENOM, San Diego, CA) at the Murdoch Children's Research Institute, Melbourne, Victoria, Australia. Briefly, 10 ng of genomic DNA was amplified using polymerase chain reaction. A MassEXTEND reaction was initiated by adding a Hotstart DNA polymerase (Bioline, Taunton, MA) and a common primer that allowed 1 base pair primer extension of either allele at the polymorphic site using the designed primers. Deoxynucleotides incorporated at the polymorphic site were terminated with the incorporation of a di-deoxynucleotide, and excess nucleotides were removed through the use of shrimp alkaline phosphatase. Using this approach, 2 allele-specific products of different mass were generated. Samples were conditioned using SpectroCLEAN (Sequenom, San Diego, CA) resin to remove excess salt that might interfere with MALDI-TOF analysis. Each sample was transferred from a 384-well microtiter plate and spotted onto the pad of a 384 SpectroCHIP II microarray. The SpectroCHIP II was placed into the MALDI-TOF and genotypes were simultaneously called in real time with SpectroTYPERTM software.

Statistical Analyses

The nongenetic covariates that were used in the analysis were baseline VA, age, sex, smoking status, lesion type, and CNV size. In addition, the 17 examined SNPs were included. The outcome of interest was change in VA from baseline to 12 months. We used a linear regression model to determine the association of nongenetic covariates with mean change in VA at 12 months, and results were presented as the regression coefficients (β) with 95% confidence interval (CI) and *P* value. When investigating the role of each covariate, we adjusted the results for all other variables. For genetic analysis, we chose a standard recessive genetic model in which the SNP covariates were converted to contain a zero for an individual, where there was only 1 or no minor allele present and 1 if 2 copies of minor allele present. If genotype data were missing for a patient (<3% of patients had missing genotype data for each SNP), that individual was not included in the analysis of that particular SNP.

For the primary analysis, we constructed a generalized linear model to investigate the association between the genetic variants of each SNP and mean change in VA after 12 months of treatment. The analysis was on a SNP-by-SNP basis, in which the statistical model was fitted for each SNP independently of the other SNPs, while adjusting for age, sex, baseline VA, smoking status, lesion type, and CNV size. The results were presented as mean change in VA for different variants of each SNP and standard error of the mean. Because we simultaneously considered 17 SNPs, there was a need to account for multiple testing. Using the Bonferroni procedure, when performing q tests, we rejected the null hypothesis at some level α, only if the associated *P* value was less than α/q. It is conventional to choose $\alpha = 0.05$, and in this case $q = 17$, meaning that *P* values needed to be <0.0029 to be considered significant.

For the secondary analysis, we investigated the role of genetic variants of the significant SNPs from our primary analysis (the SNPs significantly associated with mean change in VA at 12 months) in the likelihood of the patients losing ≥15 letters from baseline after 12 months. We used ordinary logistic regression modeling, where the outcome was either zero if the patient lost ≥15 letters from baseline after 12 months or 1 if the patient

Table 1. Patient Demographics, Study Eye Characteristics, and Treatment Details

Sex, n (%)	
Male	82 (36.6)
Female	142(63.4)
Age (yrs)	
Mean ± SD	78±6.7
Range	54–93
Baseline visual acuity* (ETDRS letter score)	
Mean ± SD	51±16.8
Range	3–80
Type of lesion, n (%)	
Predominantly classic	52 (24.8)
Nonpredominantly classic	158 (75.2)
Missing data	14 (6)†
Size of choroidal neovascularization, n (%)	
≤2 optic-disc areas‡	131(63.6)
>2 optic-disc areas‡	75 (36.4)
Missing data	18 (8)†
Injections in 12 months (n)	
Mean ± SD	6.4±2.3
Range	3–12

ETDRS = Early Treatment Diabetic Retinopathy Study; SD = standard deviation.
*Measured with ETDRS charts at 4 m.
†Missing data were not included in calculation of the percentages.
‡One optic-disc area is equal to 2.54 mm^2, based on optic-disc diameter of 1.8 mm.

Table 2. Baseline Demographics and Study Eye Characteristics on Mean Change in Visual Acuity after 12 Months of Anti-Vascular Endothelial Growth Factor Treatment

Characteristic	β* (95% CI)	*P* value
Age	−0.231 (−0.522 to 0.060)	0.119
Sex		
Female vs. male†	+0.324 (−3.778 to 4.426)	0.876
Smoking status		
Non/ex-smokers vs. current smokers†	+1.014 (−5.379 to 7.408)	0.755
Lesion type		
Predominantly classic vs. others‡	+1.030 (−3.770 to 5.830)	0.673
CNV size		
≥2 disc areas vs. <2 disc areas§	−5.873 (−10.047 to −1.699)	0.006
Baseline VA	−0.337 (−0.458 to −0.216)	<0.001

CI = confidence interval; CNV = choroidal neovascularization; VA = visual acuity.
*Coefficient of the linear regression model.
†Category of reference.
‡Minimally classic or occult lesions. This was used as the category of reference.
§One optic-disc area is equal to 2.54 mm^2, based on optic-disc diameter of 1.8 mm.

avoided a loss of ≥15 letters after 12 months treatment. The results were adjusted for age, sex, baseline VA, smoking status, lesion type, and CNV size, and presented as odds ratio (OR) and 95% CI. The statistical analyses were performed in SPSS version 19.0.1 (SPSS Inc, Chicago, IL).

Results

Two hundred twenty-four patients met the inclusion/exclusion criteria and were recruited into this study and monitored for 12 months. Mean age ± standard deviation SD) of the patients was 78±6.6 years and mean ± SD VA at baseline was 51±16.8 ETDRS letter scores (approximate Snellen equivalent of 20/100). Mean change ± SD in VA from baseline after 12 months was 3.2±14.9 letters, and the patients received on average 6.4±2.3 injections over 12 months. Of the patients, 24.8% had predominantly classic lesions and 75.2% had nonpredominantly classic (minimally classic or occult) lesions. Patient demographics, study eye characteristics, and treatment details are presented in Table 1.

Linear regression modeling examined the role of age, sex, lesion type, CNV size, smoking status, and baseline VA in the outcome of treatment at 12 months. Only baseline VA and CNV size were significantly associated with mean change in VA from baseline at 12 months (Table 2). The regression model predicted that patients with a lower baseline VA and smaller CNV size were more likely to show a greater increase in VA after 12 months of treatment, as indicated by the β coefficient (Table 2).

Genetic Analysis

A general linear modeling statistical test with a SNP-by-SNP approach adjusted for age, sex, baseline VA, lesion type, CNV size, and smoking status and a standard recessive genetic model were used to determine the association of each SNP with mean change in VA at 12 months. Table 3 presents the results for all 17 SNPs. SNP rs11200638 - *HTRA1* promoter SNP (P = 0.001)—and SNP rs10490924 (*A69S*) in *LOC387715/ARMS2* (P = 0.002) were the only SNPs to reach statistical significance

Table 3. Assessment of 17 Single Nucleotide Polymorphisms (SNPs) from Known Age-Related Macular Degeneration–Associated Risk Genes With Mean Change in Visual Acuity After 12 Months of Anti-Vascular Endothelial Growth Factor Treatment

Gene	SNP	Genotype	N	Mean VA Change at 12 Months	SE	P
HTRA (promoter SNP)	rs11200638	GG or AG	163	5.1	3.7	0.001
		AA	47	−2.9	3.8	
LOC387715/ARMS2	rs10490924 (A69S)	GG or GT	162	5.2	3.7	0.002
		TT	44	−2.6	3.8	
	rs3793917	GG or GC	151	4.7	3.6	0.005
		CC	41	−2.5	3.8	
CFH	rs800292	CC or TC	185	2.0	3.6	0.05
		TT	5	14.1	7.1	
	rs3766404	TT or TC	174	2.9	3.6	0.84
		CC	1	0.0	14.6	
	rs1061170	CC or TC	175	1.9	3.6	0.84
		TT	36	1.3	4.5	
	rs2274700	CC or TC	181	2.1	3.5	0.22
		TT	8	8.2	6.2	
	rs393955	GG or GT	177	2.1	3.6	0.96
		TT	33	2.0	4.5	
CFHR1-5	rs10922153	GG or GT	158	3.2	3.5	0.56
		TT	25	1.3	4.6	
	rs16840639	TT or TC	182	2.5	3.6	0.34
		CC	1	15.7	14.5	
	rs6667243	TT or TC	153	1.3	3.6	0.94
		CC	30	1.5	4.4	
	rs1853883	CC or GC	171	2.0	3.7	0.98
		GG	32	1.9	4.4	
C2	rs547154	CC or AC	175	1.9	3.6	0.50
		AA	2	−4.6	10.3	
C3	rs2230199	CC or GC	195	0.8	3.7	0.13
		GG	13	4.0	3.8	
	rs1047286	CC or CT	170	2.3	3.5	0.55
		TT	10	5.0	5.7	
CFB	rs641153	GG or GT	158	3.2	3.5	0.56
		TT	25	1.3	4.6	
F13B	rs6003	AA or GA	184	2.1	3.6	0.58
		GG	1	9.7	14.3	

CFH = complement factor H; *CFHR* = complement factor H related; *C2* = complement component 2; *C3* = complement component 3; *CFB* = complement factor B; *F13B* = factor 13B; *HTRA1* = high temperature requirement factor A1; *LOC387715/ARMS2* = Age-Related Maculopathy Susceptibility 2; SE = standard deviation; VA = visual acuity.
Results obtained after adjusting for age, sex, smoking status, baseline visual acuity, lesion type, and lesion size; accepted $P \leq 0.0029$ after multiple correction.

(accepted P value for significance = 0.0029 after multiple correction) for the outcome at 12 months. SNP rs3793917 in *LOC387715/ARMS2* also showed nominal significance with P = 0.005 (Table 3), but no other SNPs attained significance (Table 3).

The minor allele frequency of both SNP rs11200638 and SNP rs10490924 (A allele in SNP rs11200638 and the T allele in SNP rs10490924) was 0.44 in our cohort. The mean ± SD change in VA from baseline in 47 patients homozygous for the A risk allele (AA genotype) at SNP rs11200638 in the *HTRA1* gene was −2.9±15.2 letters after 12 months compared with +5.1±14.1 letters in patients with either the AG or GG genotypes at this SNP (Fig 1). The AG or GG genotype group consisted of 163 patients and a breakdown of this group indicated that those individuals with the AG genotype (92 patients) had a mean change ± SD in VA after 12 months of +4.5±12.9 letters, whereas the 71 patients with the GG genotype had a mean change ± SD in VA after 12 months of +5.8±15.5 letters. Although people with 2 copies of the wild-type allele (GG genotype) at this SNP responded slightly better than those with only 1 copy of the wild-type allele (AG genotype), a recessive model comparing the VA outcome of patients homozygous for the minor allele (AA genotype) with that of the patients with AG or GG genotypes provided the best fit in terms of association of genetic variants of SNP rs11200638 with a poor outcome to treatment. Similarly, patients homozygous for the T risk allele (TT genotype) at SNP rs10490924 in the *LOC387715/ARMS2* gene had a mean ± SD change in VA from baseline of −2.6±15.4 letters compared with +5.2±14.2 letters in those with GT or GG genotypes. In addition, SNP rs3793917 in the *LOC387715/ARMS2* gene reached a nominal significance (P = 0.005) for worse outcome to anti-VEGF treatment after 12 months. These findings are perhaps not surprising given the high correlation between these 3 SNPs with r^2 value = 0.92 for each pair of these SNPs in our cohort (Fig 2). Table 4 (available at http://aaojournal.org) presents the VA outcomes of all genotype variants of SNPs rs11200638, SNP rs10490924, and SNP rs3793917 in our patients.

We also investigated whether homozygote genotypes for the risk alleles at any of the 3 significant SNPs were associated with increased risk of patients losing ≥15 letters of VA from baseline after 12 months of treatment. After adjustment for age, sex, baseline VA, smoking status, lesion type, and CNV size, patients with the AA genotype at SNP rs11200638 in the *HTRA1* gene were 3.7 times (OR, 3.73; 95% CI, 1.51−9.26) more likely to lose ≥15 letters after 12 months treatment compared with those with either the AG or GG genotypes (P = 0.004; Table 5). The SNPs rs10490924 and rs3793917 also showed similar size ORs of 3.01 and 2.93, respectively, and were significant, considering the standard P value for significance of p = 0.05 (Table 5).

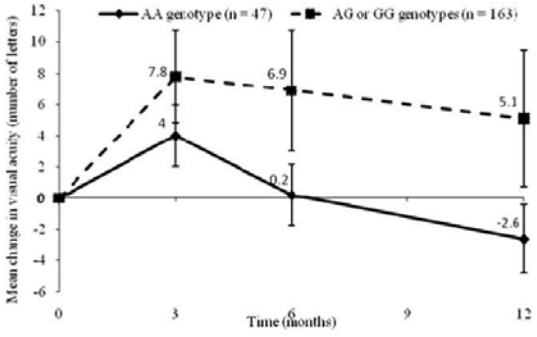


Figure 1. Comparison of mean change in visual acuity (VA) from baseline in patients harboring the AA risk genotype at single nucleotide polymorphism (SNP) rs11200638 with that of patients with the AG or GG genotypes at this SNP over the 12-month course of study. Vertical lines represent 95% confidence interval of mean, indicating that the difference in mean change in VA between these 2 groups was significant at 6 and 12 months.

Discussion

In this study, we have shown for the first time a strong genetic association for homozygote risk alleles at SNP rs11200638 (*HTRA1* promoter SNP) and SNP rs10490924 (*A69S*) in the *LOC387715/ARMS2* gene with poor outcome of intravitreal anti-VEGF injections in treatment-naïve patients with neovascular AMD. We also found a borderline association between homozygote risk alleles at SNP rs3793917 in the *LOC387715/ARMS2* gene and poor response to this treatment.

The risk alleles rs10490924 and rs11200638 have also been associated with neovascular AMD in several studies involving different populations.[18,28-32] Sobrin et al[33] examined the variants of 115 SNPs in 4 cohorts comprising a total of 3937 patients of European ancestry with CNV or geographic atrophy. They reported that the T allele (risk allele) at SNP rs10490924 was significantly associated with an increased risk of CNV compared with geographic atrophy.[33]

With regard to the association of variants in *HTRA1/ARMS2* locus and anti-VEGF treatment outcome, the results of previous studies are inconsistent. In a small study of 90 patients, Teper et al[24] reported that patients with TT genotypes at SNP rs10490924 (*LOC387715/ARMS2*) gained 1.35 letters after 12 months of ranibizumab treatment, whereas patients with the GT and GG genotypes at this SNP gained 4.33 and 7.16 letters, respectively, after 12 months. On the basis of these results, they concluded that patients homozygous for the risk allele at SNP rs10490924 (*LOC387715/ARMS2*) showed a worse outcome with ranibizumab treatment.[24]

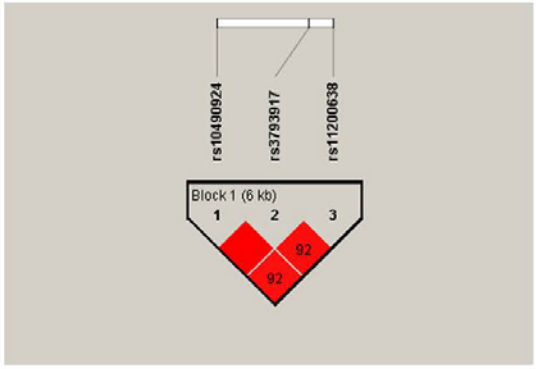


Figure 2. Linkage disequilibrium (LD) plot of the 3 examined single nucleotide polymorphisms across the *ARMS2*−*HTRA1* gene region with r^2 values (a measure of LD) illustrated.

Table 5. Role of 3 Single Nucleotide Polymorphisms (SNPs) in the *HTRA1* and *LOC387715/ARMS2* Genes to Assess the Likelihood of Patients Losing ≥15 Letters of Vision After 12 Months of Anti-Vascular Endothelial Growth Factor Treatment

Gene	SNP	Genotype	N	Loss of ≥15 Letters at 12 Months, n (%)	OR (95% CI)	P
HTRA1 (promoter SNP)	rs11200638	GG or AG	163	15 (9.3)	1.00	0.004
		AA	47	13 (27.7)	3.73 (1.51–9.26)	
LOC387715/ARMS2	rs10490924	GG or GT	162	16 (9.9)	1.00	0.02
		TT	44	11 (25.6)	3.01 (1.18–7.71)	
	rs3793917	GG or GC	151	15 (9.9)	1.00	0.03
		CC	41	10 (25.0)	2.93 (1.09–7.87)	

CI = confidence interval; *HTRA1* = high temperature requirement factor A1; *LOC387715/ARMS2* = Age-Related Maculopathy Susceptibility 2; OR = odds ratio.
Results obtained after adjusting for age, sex, smoking status, baseline visual acuity, lesion type, and lesion size; accepted $P \leq 0.05$.

However, the significant weaknesses of this study make the interpretation of their results very difficult. These included its relatively small study size, but most important, 74 of 90 patients (82.2%) were not treatment naïve and had received a large variety of prior treatment modalities including photodynamic therapy, ranibizumab, bevacizumab, pegaptanib, steroids, and combination therapy before the onset of the study.[24] However, in a clinical trial by McKibbin et al,[23] the risk allele (A allele) at *HTRA1* promoter SNP (rs11200638) was reported to be associated with a better VA outcome for ranibizumab treatment in a Caucasian, treatment-naïve cohort with 104 patients. This is in contrast with our findings.[23] The CATT study group also recently reported that they found no association between SNP rs10490924 (*ARMS2*) or SNP rs11200638 (*HTRA1*) and the outcome of ranibizumab or bevacizumab treatment in neovascular AMD (Hagstrom SA. Pharmacogenetics of Anti-VEGF Therapy in the Comparison of AMD Treatments Trials [CATT]. Paper presented at: ARVO Annual Meeting, May 8, 2012, Fort Lauderdale, Florida). They recruited 834 patients, of multiethnic origins, from 43 CATT clinical centers in the United States. (Large numbers of clinics could add more measurement variability compared with our single-center study of a purely Caucasian cohort.) In their preliminary report, it was not possible to determine the covariables adjusted for in their analyses. Strong association of SNPs rs11200638 and rs10490924 with the risk of CNV and a poor response to anti-VEGF treatment in patients with CNV secondary to AMD suggests an underlying biological mechanism. SNP rs11200638 is located on the putative promoter region of *HTRA1* gene at chromosome region 10q26.[32] In a study by Yang et al,[18] the AA genotype at SNP rs11200638 was associated with higher *HTRA1* mRNA levels in lymphocytes of AMD patients as well as a higher mean HTRA protein level in retinal pigment epithelium of AMD donor eyes compared with normal controls with the GG genotype. The enhanced transcription of *HTRA1* in patients with the AA genotype at SNP rs11200638 was also reported in another study.[32] The HTRA1 protein, expressed in human retina, is a heat shock protein in the serine protease family and seems to regulate degradation of the extracellular matrix proteoglycans.[18,34] As a result, overexpression of HTRA1 may affect the integrity of Bruch's membrane and potentiate the invasion of choroidal blood vessels across the extracellular matrix.[18] In addition, HTRA1 inhibits transforming growth factor-β, which regulates angiogenesis.[35] In a recent study of a large cohort of Caucasian patients, Zhang et al[36] suggested that the *HTRA1* gene plays a critical role in angiogenesis through transforming growth factor-β family member growth differentiation factor 6 (GDF6).[3]

With regard to the possible biological mechanism for the effect of SNP rs10490924 (*A69S*) in the treatment outcome, localization of ARMS2 protein to the outer membrane of mitochondria suggests that the effect could be through oxidative damage of photoreceptors and retinal pigment epithelium.[16] A decrease in mitochondrial number and size has been reported in AMD retinas.[37]

Loss of integrity of Bruch's membrane and/or angiogenesis subsequent to *HTRA1* overexpression, driven by the AA genotype at the *HTRA1* promoter SNP or oxidative pathways mediated by *LOC387715/ARMS2 A69S* variants, may very well contribute to the underlying biological mechanisms for a poor response to intravitreal anti-VEGF treatment.

The strengths of our study include its prospective design and the relatively large cohort of consecutive patients, participants only of known Caucasian ancestry, who were recruited from a single center. In addition, all patients were treatment naïve and minor allele frequencies of the examined SNPs were comparable with those of other AMD cohorts, including our own previous study.[33,38] Patient demographic and baseline study eye characteristics were adjusted for in our statistical model.

The weakness of this study was in its PRN treatment protocol, with the decision to retreat at the discretion of the clinicians based on retreatment criteria at each follow-up visit. However, the mean number of injections required over the 12-month treatment course of this study was similar in patients homozygous for the risk allele and those with <2 copies of the risk allele at the 3 significant SNPs, with, on average, 6 injections per person over 12 months in all different groups. All clinicians were masked to patients' genotype. This makes it unlikely that a different number of injections could be a possible confounding factor in our findings.

This study of 224 patients is of a comparable size to most other single-center studies regarding genetic studies and

anti-VEGF treatment outcome. It suggests a strong pharmacogenetic mechanism for the 10q26 locus in response to intravitreal anti-VEGF treatment in neovascular AMD. This will help us move toward an individualized treatment protocol to achieve optimal response with the fewest possible injections or more intensive treatment, based on genotype, with increased cost effectiveness of this novel treatment in certain groups of patients.

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Variants in the *VEGFA* Gene and Treatment Outcome after Anti-VEGF Treatment for Neovascular Age-related Macular Degeneration

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Purpose: To determine the association of genetic variants of the *VEGFA* gene with outcome of anti-vascular endothelial growth factor (VEGF) treatment in neovascular age-related macular degeneration (AMD).

Design: A prospective cohort study.

Participants: We included 201 consecutive patients receiving anti-VEGF injections for neovascular AMD.

Methods: Patients were followed over 12 months. They were treated with 3 initial monthly ranibizumab or bevacizumab injections. Thereafter, the decision to retreat was made by clinicians at each follow-up visit on the basis of retreatment criteria. Seven tagged single nucleotide polymorphisms (tSNPs) in the *VEGFA* gene were selected and examined. Multivariate data analysis was used to determine the role of each tSNP in treatment outcome.

Main Outcome Measures: The influence of selected *VEGFA* tSNPs on visual acuity (VA) outcome at 6 months.

Results: Mean baseline VA was 51±17 Early Treatment Diabetic Retinopathy Study (ETDRS) letter scores. Overall, the mean change in VA from baseline was +6.5±12, +4.4±13.4, and +2.3±14.6 letters at 3, 6, and 12 months, respectively. The tSNP rs3025000 was the only SNP significantly associated ($P<1 \times 10^{-4}$) with visual outcome at 6 months with multiple correction. The presence of the T allele (TC or TT genotypes) at this tSNP predicted a better outcome of +7 letters at 6 months compared with the CC genotype. In a subgroup analysis, presence of the T allele predicted a significantly higher chance of the patients belonging to the responder group (gain of ≥5 letters from baseline) after 3, 6, and 12 months treatment (odds ratio, 2.7, 3.5, and 2.4; 95% confidence interval, 1.46–5.07, 1.82–6.71, and 1.27–4.57, respectively) than any other outcome group.

Conclusions: Pharmacogenetic association with anti-VEGF treatments may influence the visual outcomes in neovascular AMD. In patients with the T allele in tSNP rs3025000, there was a significantly better visual outcome at 6 months and a greater chance of the patients belonging to the responder group with anti-VEGF treatment at 3, 6, and 12 months. The VA outcomes of patients harboring the T allele at SNP rs3025000 were comparable with those of the pivotal clinical trials but with fewer injections, making the treatment perhaps more cost effective in certain subgroups of patients.

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Vascular endothelial growth factor (VEGF) inhibition with the intravitreal anti-VEGF monoclonal antibodies bevacizumab and ranibizumab has revolutionized the treatment of neovascular age-related macular degeneration (AMD).[1–3] The international randomized clinical trials (RCTs) of ranibizumab—ANCHOR (*AN*ti-VEGF antibody for the treatment of predominantly classic *CHOR*oidal neovascularization in age-related macular degeneration) and MARINA (*M*inimally classic/occult trial of the *A*nti-VEGF antibody *R*anibizumab *I*n the treatment of *N*eovascular *A*ge-related macular degeneration)—have reported that, with monthly ranibizumab injections, 30% to 40% of patients gained >15 Early Treatment Diabetic Retinopathy Study (ETDRS) letters, 5% to 10% lost >15 letters despite treatment, and the rest maintained vision.[2,3] Year 1 results of the CATT (Comparison of Age-Related Macular Degeneration Treatments Trials) study showed that bevacizumab and ranibizumab had similar effects on best-corrected visual acuity (BCVA) when administered under the same treatment protocol.[4]

The SUSTAIN study, which investigated a different ranibizumab treatment protocol with a fixed monthly dosing

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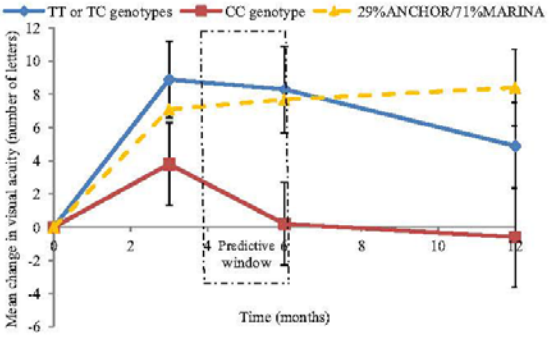


Figure 1. Comparison of the mean change in visual acuity over 12 months in patients with and without the T allele at SNP rs3025000 in the vascular endothelial growth factor A (*VEGFA*) gene. ANCHOR = ANti-VEGF antibody for the treatment of predominantly classic CHORoidal neovascularization in age-related macular degeneration; MARINA = Minimally classic/occult trial of the Anti-VEGF antibody Ranibizumab In the treatment of Neovascular Age-related macular degeneration; SNP = single nucleotide polymorphism.

regimen for 3 months, followed by a flexible, "as required" treatment for 9 months, showed that 53% of patients gained and maintained vision, 21% gained but did not maintain, and 26% did not have any gain in BCVA.[5] The SUSTAIN results showed that the outcome of the treatment in the first 3- to 5-month period could predict the outcomes at year 1 so that responder types could be recognized.[5] In the EXCITE study, which compared the outcome of 3 different treatment regimens—quarterly 0.5 mg ranibizumab, quarterly 0.3 mg ranibizumab, and monthly 0.3 mg ranibizumab after 3 initial loading doses—a similar divergence of response was noted at month 4.[6] Thus, it seems that the visual outcome of the treatment in the first 4 to 6 months can predict the outcome at year 1. This 4- to 6-month treatment window has been coined a predictive window (Figure 1).

The outcome after the first 6 months of treatment may be more informative than longer-term outcomes when investigating the effect of genotype on response to treatment. The shorter time period avoids issues of the variable treatment intervals that are likely to occur at later time points when anti-VEGF treatment decisions are left up to the treating clinicians. Using a 6-month time point for the primary endpoint of the visual acuity (VA) outcome gives the best opportunity, we believe, to examine possible genetic influences on the outcome because it avoids the effect of confounding variables that impact the outcome with longer durations.

Recent research on outcome determinants has focused on the role of known AMD risk-associated genes on the outcome of anti VEGF treatment for neovascular AMD. Complement factor H (*CFH*), age-related macular susceptibility 2 (*ARMS2*), in particular the *A69S* variant, and high-temperature requirement A-1 (*HTRA1*) genes have been reported to show some association with VA outcome after treatment with bevacizumab or ranibizumab.[7–9] In a prospective study by Teper et al,[8] patients homozygous for the T risk allele at *A69S* of the *ARMS2* gene showed a poor BCVA response to a variable dosing treatment regimen with ranibizumab. However, at the Association for Research in Vision and Ophthalmology 2012 meeting, the CATT study group reported that they found no association between single nucleotide polymorphisms (SNPs) rs1061170 (*CFH*), rs10490924 (*ARMS2*), rs11200638 (*HTRA1*), and rs2230199 (*C3*) and the outcome of ranibizumab or bevacizumab treatment in neovascular AMD (Hagstrom SA. Pharmacogenetics of Anti-VEGF Therapy in the Comparison of AMD Treatments Trials (CATT). Paper presented at: Association for Research in Vision and Ophthalmology Annual Meeting, May 08, 2012; Florida).

We have previously reported that patients with the apolipoprotein E (*ApoE*) ε4 allele had significantly better BCVA outcomes after anti-VEGF treatment than those without this allele.[10]

An endothelial cell-specific mitogen, VEGF-A triggers angiogenesis and is a mediator of vascular permeability.[11] It initiates and promotes pathologic choroidal neovascularization (CNV) processes in AMD.[12] The *VEGFA* gene, located on chromosome 6p 21.1 (NM_003376) (http://www.ncbi.nlm.nih.gov/nuccore; accessed April 20, 2011), consists of 8 exons and, as a result of alternative splicing, it produces 4 major VEGF-A isoforms—VEGF-A 121, VEGF-A 165, VEGF-A 189, and VEGF-A 206.[13] Ranibizumab and bevacizumab induce their therapeutic effects via blocking all VEGF-A isoforms.[1,14] We investigated whether different SNP variations in the *VEGFA* gene could result in different outcomes of anti-VEGF treatment.

Several SNPs in the *VEGFA* gene have been investigated for their influence on anti-VEGF treatment outcomes, with inconclusive results. One study has reported a trend toward a better outcome after 6 months' treatment with ranibizumab in those harboring the TC or CC genotypes, compared with those having the TT genotype at SNP rs1413711 in the *VEGFA* gene.[7] However, other investigators have not found any association between different genotypes at this SNP (rs1413711) and VA outcome after treatment.[15] A Japanese study has reported that the G allele at SNP rs699946 in the *VEGFA* gene is associated with a better response to bevacizumab treatment in patients with neovascular AMD.[16]

In this study, we investigated whether certain SNPs in the *VEGFA* gene are associated with the outcome of anti-VEGF treatment in patients with neovascular AMD collected through a single center.

Methods

Study Design and Eligibility

Our study population was prospectively recruited from the medical retina clinics of The Royal Victorian Eye and Ear Hospital (RVEEH) from 2006 to 2010. This study was approved by the Human Research and Ethics Committee of the RVEEH. Research adhered to the tenets of the Declaration of Helsinki, and the participants provided written informed consent before participation.

Patient Selection

Inclusion criteria were patients aged >50 years with active subfoveal CNV due to AMD who received intravitreal bevacizumab or ranibizumab in RVEEH medical retina clinics since 2006.

Patients were identified by medical retina specialists, experienced in the management of AMD, on the basis of ophthalmic examination, fundus fluorescein angiography, fundus photography, and time-domain optical coherence tomography (OCT). Establishing the diagnosis of active CNV required the presence of subfoveal leakage, as seen on fundus fluorescein angiography. All patients with subfoveal CNV were included and followed for 12 months. One eye was randomly determined as the study eye if both eyes were eligible (30 participants underwent bilateral treatment).

Exclusion criteria were (1) CNV secondary to non-AMD conditions such as angioid streaks, degenerative myopia, central serous retinopathy, or hereditary retinal disorders; (2) laser photocoagulation or photodynamic therapy before anti-VEGF administration; (3) any serious adverse events over the treatment course; and (4) and non-Caucasian ancestry.

Data Collection and Follow-up

Data pertaining to patient demographics, medical history, VA, and treatment scheduling were collected after consent was obtained. Experienced examiners measured VA using ETDRS charts at 4-m distance at each visit. A peripheral blood sample was collected from each patient for DNA extraction. At each appointment, VA was reassessed and patients underwent a dilated fundus examination and a time-domain OCT imaging with Stratus OCT version 5.0.1 (Carl Zeiss Meditec, Dublin, CA; or Cirrus HD-OCT version 6.0.0.599, Carl Zeiss Meditec).

All angiograms were examined by medical retina specialists to determine the lesion type and measure CNV size in comparison with optic disc area using the middle phase frame of the angiogram.

Treatment

Patients were treated with a dose of 0.5 mg intravitreal ranibizumab (Lucentis; Novartis Pharma AG, Basel, Switzerland) or 1.25 mg bevacizumab (Avastin; Roche, Basel, Switzerland). The majority of patients were treated with ranibizumab only during the course of treatment. However, 39 patients (19.4%) received a single injection of bevacizumab during their baseline visit while waiting for government approval for ranibizumab. In addition, during the course of treatment, bevacizumab was used in a follow-up visit if no approved ranibizumab prescription was available (<5% of total injections). All patients received 3 initial monthly injections followed by a flexible (as required) period. The decision to re-treat in the flexible period was at the discretion of the treating retinal specialist at each follow-up visit on the basis of re-treatment criteria, including VA loss of >5 letters, increased central retinal thickness of >100 μm, or the presence of retinal fluid on OCT (intraretinal or subretinal) or ophthalmic examination findings of new or persistent hemorrhage. Extension of 2 weeks was considered for the subsequent clinic visit if the clinical situation was stable and OCT was free of intraretinal or subretinal fluid.

Genotyping

Briefly, 10 ng of genomic DNA were amplified using the polymerase chain reaction in a multiplex reaction using Hotstart taq polymerase (Bioline, London, UK). A MassEXTEND reaction was undertaken using the designed primers. Samples were spotted onto a 384 SpectroCHIP II microarray and genotyping was performed on the MassArray platform (SEQUENOM, San Diego, CA) at the Murdoch Children's Research Institute (Melbourne, Victoria, Australia).

Seven SNPs—rs3024994, rs3025000, rs3025042, rs3025047, rs3025035, rs3025030, and rs3025010—in the *VEGFA* gene were identified through the selection of tag SNPs (tSNPs) via the use of the International HapMAP Project (http://www.hapmap.org/; accessed July 20, 2010). The tSNPs were selected using the pairwise algorithm and a CEU (Utah residents with ancestry from northern and western Europe) population. A strong linkage disequilibrium tagging criteria of r^2>0.8 was used and a minor allele frequency of 0.01 was chosen. The HapMAP data was sourced from NCBI Build35. Table 1 and Figure 2 (available at http://aaojournal.org) present the details of the assessed SNPs and the linkage disequilibrium plot for our cohort, respectively.

Statistical Analysis

The nongenetic covariates that were used in the analysis were baseline VA, age, sex, smoking status, lesion type, and CNV size. In addition, the 7 tSNPs from the *VEGFA* gene were also included. The outcomes of interest were change in VA from baseline at 3, 6, and 12 months. We chose a standard dominant genetic model in which the SNP covariates were converted to contain a zero for an individual, where there was no minor allele present, and 1 if there was ≥1 minor allele present. There was a small proportion of missing data for the SNP covariates (missing data were <3% for each SNP) and this was imputed using the "*k*-nearest neighbors" approach, with $k = 1$ (i.e., using the value of the genetically closest neighbor), and using the Hamming distance, which is appropriate for genetic data (removing patients with missing data for each SNP did not significantly change the results).

For the primary analysis, we used least-squares regression modeling. The analysis was on a SNP-by-SNP basis, in which a regression model was fitted for each SNP independently of the other SNPs, while adjusting for age, sex, baseline VA, smoking status, lesion type, and CNV size. A t test was then used to compute a P value of association for that particular SNP. Because we simultaneously considered 7 hypotheses, there was a need to account for multiple testing. Using the Bonferroni procedure, when performing q tests, we rejected the null hypothesis at some level α, only if the associated P value was less than α/q. It is conventional to choose $\alpha = 0.05$, and in this case $q = 7$, meaning that P values needed to be <0.0071 to be considered significant.

For the secondary analysis, we defined the level of response. We used a margin of 5 letters for our secondary analysis, similar to the EXCITE study group, which used a margin of 5 letters to compare different treatment protocols.[6] Therefore, a *responder* was defined as a patient with a gain of ≥5 ETDRS letters from baseline, *stable* was defined as a gain or loss of <5 letters, and *nonresponder* was defined as a patient with a loss of >5 letters. We used ordinary logistic regression modeling where the outcome was either zero if the patient was a responder or 1 if the patient was in the stable/nonresponder (stable or nonresponder) group. The analysis was also performed in a SNP-by-SNP fashion, adjusting for age, sex, baseline VA, smoking status, lesion type, and CNV size. Because the patients in the study did not receive exactly the same number of injections (the result of the as-needed treatment regimen), we repeated all the analyses, adjusting the results for the number of injections at each time point in the statistical model.

Results

The study population comprised 201 patients who met the eligibility criteria. Patient demographics and baseline characteristics

are presented in Table 2 (available at http://aaojournal.org). Mean patient age was 78±7 years with a mean baseline VA of 51±17 ETDRS letter scores (approximate Snellen equivalent of 6/30). Overall, mean change in VA was +6.5±12.0, +4.4±13.4, and +2.3±14.6 letters at 3, 6, and 12 months, respectively. On average, patients received 4.3±1.0 injections in the first 6 months and 2.4±1.4 injections in the second 6 months of the treatment course. Univariate regression analysis of age, sex, lesion type, CNV size, smoking status, and baseline VA showed that baseline VA was the only variable with significant contribution to the VA outcome at 3, 6, and 12 months. For the impact of baseline VA, the regression model predicted that with each increment of 1 letter in the baseline VA, there would be, on average, a 0.33 ($P<10^{-10}$), 0.32 ($P<10^{-8}$), and 0.31 ($P<10^{-7}$) letters greater decline in VA change after 3, 6, and 12 months, respectively (Table 3, available at http://aaojournal.org). Accordingly, patients with better baseline VA were less likely to show VA gains compared with patients with lower baseline VA; patients with poorer baseline VA were less likely to further lose VA, the so-called floor and ceiling effects as observed in the subgroup analysis of ANCHOR and MARINA studies.[17,18] However, the final VA achieved after 12 months was higher in patients with better baseline VA. For example, the mean change in VA and the final VA for the patients with baseline VA of <55 ETDRS letter scores were +6 and 50 ETDRS letter scores after 12 months treatment compared with −2 and 65 ETDRS letter scores in patients with a baseline VA of ≥55 ETDRS letter scores. Lesion type was also significantly associated with the VA outcome; patients with predominantly classic lesions showed greater improvement in VA from baseline at 3 ($P = 8 \times 10^{-4}$) and 6 ($P = 5 \times 10^{-3}$) months compared with those with minimally classic or occult lesions (Table 3, available at http://aaojournal.org).

Genetic Analysis

A summary of the SNP statistics is shown in Table 4 (available at http://aaojournal.org). Ordinary least-squares regression modeling with a SNP-by-SNP approach was used to determine the role of each SNP on the outcome of the treatment. Table 5 shows the *P* values for the contribution of different SNPs to the mean change in VA from baseline at 3, 6, and 12 months after adjusting for age, sex, baseline VA, smoking status, lesion type, and CNV size. Only SNP rs3025000 attained the *P*-value requirement of 0.007 for the outcome at 6 months ($P<10^{-4}$). The β coefficient was +7.1 for SNP rs3025000 at 6 months, indicating that the presence of the minor (T) allele at SNP rs3025000 (TT or TC genotypes) led to +7 letters greater increase in the visual outcome at 6 months compared with patients with the CC genotype at this SNP. The role of this SNP in the mean change in VA from baseline at 3 and 12 months was also marginally significant ($P = 0.01$ at 3 and 12 months) after Bonferroni correction. Mean change in VA in patients with the T allele (TT or CT genotype) at SNP rs3025000 was +8.8±1.2, +8.3±1.3, and +4.9±1.3 letters after 3, 6, and 12 months, respectively, compared with +3.9±1.3, +0.2±1.3, and ×0.6±1.5 letters in patients without the T allele (CC genotype; Figure 1).

In an analysis of the subgroups of the cohort, we divided the patients into the responder (gain of ≥5 letters), stable (gain or loss of <5 letters), and nonresponder (loss of ≥5 letters) groups separately at each time point. After 3, 6, and 12 months, we had 117 (58.2%), 94 (46.8%), and 99 (49.3%) patients in the responder group and 84 (41.8%), 107 (53.2%), and 102 (50.7%) patients in the stable/nonresponder (stable or nonresponder) group, respectively. A multivariate data analysis adjusted for age, sex, baseline VA, smoking status, lesion type, and CNV size was performed to determine the association of the 7 tSNPs and the likelihood of the patients being in the responder versus the stable/nonresponder group. Table 6 presents the odds ratios (ORs) and *P* values of this analysis. The presence of the T allele (TT or TC genotypes) at SNP rs3025000 was significantly associated with the likelihood of patients to be in the responder group after 3 months (OR, 2.7; 95% confidence interval [CI], 1.46–5.07; $P<1 \times 10^{-3}$), 6 months (OR, 3.5; 95% CI, 1.82–6.71; $P<1 \times 10^{-4}$), and 12 months (OR, 2.4; 95% CI, 1.27–4.57; $P = 6 \times 10^{-3}$). Other SNPs did not show any significant association with the VA outcome.

Out of 105 patients with the presence of the T allele at SNP rs3025000, 73 (69.5%), 64 (61%), and 62 (59%) patients were in the responder group after 3, 6, and 12 months of treatment, respectively. However, in 96 patients with CC genotype at this SNP only 44 (45.8%), 30 (31.2%), and 37 (38.5%) patients were

Table 5. *P* Values for Association of Each Tag Single Nucleotide Polymorphism (tSNP) of the Vascular Endothelial Growth Factor A (*VEGFA*) Gene with Mean Change in Visual Acuity after 3, 6, and 12 Months of Anti-Vascular Endothelial Growth Factor Treatment

tSNP	rs3024994	rs3025000	rs3025010	rs3025030	rs3025035	rs3025042	rs3025047
3 months							
*P**	0.77	0.01	0.58	0.09	0.42	0.63	0.30
β†	0.79	3.96	−0.93	3.01	1.88	2.30	−4.50
SE	2.76	1.58	1.71	1.75	2.31	4.72	4.35
6 months							
*P**	0.25	**$<1 \times 10^{-4}$**	0.88	0.02	0.57	0.08	0.87
β†	3.51	7.11	−0.29	4.56	1.44	9.10	−0.80
SE	3.02	1.69	1.88	1.91	2.55	5.15	4.79
12 months							
*P**	0.12	0.01	0.12	0.07	0.28	0.19	0.67
β†	5.10	4.57	−3.16	3.86	−3.01	7.37	−2.22
SE	3.30	1.91	2.05	2.11	2.79	5.68	5.26

SE = standard error.
Least-squares regression modeling adjusted for age, sex, smoking status, baseline visual acuity, lesion type, and choroidal neovascularization size was used for the analysis.
*Significance accepted at P<0.007 (bold).
†β is the linear regression coefficient and represents the change in visual acuity in the presence of the minor allele (homozygous or heterozygous) compared with homozygous major allele at each tSNP.

Table 6. Odds Ratios and *P* Values for the Association of Each Tag Single Nucleotide Polymorphism (tSNP) of the Vascular Endothelial Growth Factor A Gene with the Likelihood of a Patient Being in the Responder versus Stable/Nonresponder Group after Anti-Vascular Endothelial Growth Factor Treatment

		3 Months		6 Months		12 Months	
tSNP	Genotype	*OR (95% CI)*	P *value**	*OR (95% CI)*	P *value**	*OR (95% CI)*	P *value**
rs3024994	CC	1		1		1	
	TT or TC	0.92 (032–2.60)	0.87	2.87 (0.92–8.95)	0.06	1.79 (0.60–5.40)	0.29
rs3025000	CC	1		1		1	
	TT or TC	2.72 (1.46–5.07)	**$<1 \times 10^{-3}$**	3.49 (1.82–6.71)	**$<1 \times 10^{-4}$**	2.38 (1.27–4.57)	**6×10^{-3}**
rs3025010	TT	1		1		1	
	CC or CT	0.77 (0.40–1.47)	0.42	0.80 (0.41–1.54)	0.49	0.59 (0.30–1.16)	0.12
rs3025030	GG	1		1		1	
	CC or CG	1.14 (0.58–2.23)	0.70	1.41 (0.71–2.83)	0.31	1.61 (0.80–3.21)	0.17
rs3025035	CC	1		1		1	
	TT or CT	1.24 (0.52–2.95)	0.62	1.19 (0.49–2.90)	0.69	1.11 (0.46–2.67)	0.80
rs3025047	CC	1		1		1	
	TT or TC	0.58 (0.12–2.87)	0.49	1.30 (0.26–6.42)	0.74	0.44 (0.08–2.52)	0.35

CI = confidence interval; OR = odds ratio.
Ordinary logistic regression modeling adjusted for age, sex, smoking status, baseline visual acuity, lesion type, and choroidal neovascularization size was used for the analysis. The results for SNP rs3025042 are not included because the minor allele frequency was very small and the results were highly unreliable.
*Significance accepted at $P<0.007$ (bold).

in the responder group after 3, 6, and 12 months treatment, respectively.

The flexible treatment regimen in this study could result in different numbers of injections in different genotype groups. However, the number of injections the patients with the TT or TC genotype at SNP rs3025000 received (4.1±1.1 injections in the first 6 months and 6.3±2.1 injections over 12 months) was similar to that the patients with CC genotype at this SNP received (4.1±1.2 injections in the first 6 months and 6.2±2.5 injections over 12 months). To ensure that different numbers of injections did not affect the results for any of the 7 examined tSNPs, all the multivariate analyses were repeated for each tSNP after adjusting for the number of injections. We did not observe any significant differences in the results for any of the SNPs (results not shown).

Comparison with the ANCHOR and MARINA Outcomes

The promising outcomes of the pivotal ANCHOR and MARINA studies, achieved with monthly ranibizumab injections, have been used by various clinical trials as the standard reference for best outcomes, when comparing the efficacy of different treatment protocols.[5,6] We have compared the visual outcomes of the patients harboring the minor (T) allele at SNP rs3025000 (TT or TC genotypes) and those without the minor (T) allele (CC genotype) at this SNP in the *VEGFA* gene with the outcomes of the patients who received monthly 0.5 mg of ranibizumab injections in the ANCHOR and MARINA studies. Our aim was to investigate whether patients with the TT or TC genotype at SNP rs3025000 could achieve comparable outcomes with those of the pivotal studies even with a different treatment protocol requiring fewer injections. Mean age and baseline VA were similar in our cohort harboring the T allele at SNP rs3025000 (mean age, 78.3±6.8 years; mean baseline VA, 50.3±17.9 ETDRS letter scores), those without the T allele at this SNP (mean age, 77.8±6.8 years; mean baseline VA, 52.5±15.6 ETDRS letter scores), and the ANCHOR (mean age, 76±8.6 years; mean baseline VA, 47.1±13.2 ETDRS letter scores) and MARINA (mean age, 77±8 years; mean baseline VA, 53.7±12.8 ETDRS letter scores) studies.[2,3] We had 105 patients with TT or TC genotypes at SNP rs3025000: 31 (29.5%) with predominantly classic (the lesion type in the ANCHOR study) and 74 (70.5%) with nonpredominantly classic (the lesion type in the MARINA study) lesions.[2,3] Weighted calculation of the ANCHOR and MARINA visual outcomes for our cohort (29.5% ANCHOR/70.5% MARINA) predicts theoretical mean VA changes of 7.1, 7.7, and 8.4 letters after 3, 6, and 12 months, respectively, with monthly injections similar to those RCTs (mean VA change in the ANCHOR study × 29.5% + mean VA change in the MARINA study × 70.5%, which was used to calculate the weighted outcomes at each time point, as described by the SUSTAIN study group[5] and Gerding et al[19]). Figure 1 compares the visual outcomes of our patients with TT or TC genotypes at SNP rs3025000 with those harboring CC genotype at this SNP and the weighted ANCHOR/MARINA outcomes using 95% CI bars. The visual outcomes of those, with the TT or TC genotypes at SNP rs3025000 over the 12-month course of this study were similar to those of the weighted ANCHOR/MARINA outcomes and significantly better than the outcomes of the patients with the CC genotype at this SNP (Figure 1).

Discussion

We identified a significant association for SNP rs3025000 in the *VEGFA* gene in the outcome of anti-VEGF treatment for neovascular AMD. The presence of ≥1 copies of the minor (T) allele in this SNP predicted a clinically significant greater increase in VA of +7 letters at month 6. In addition, the presence of the minor (T) allele indicated a 2.7-, 3.5-, and 2.4-fold increase in the likelihood of being in the responder group than in the stable/nonresponder group after treatment in our cohort after 3, 6, and 12 months, respectively (Table 6). We were unable to find any other significant association between other SNPs in the *VEGFA* gene and VA outcome.

This is the first study that has investigated the role of SNP rs3025000 in the *VEGFA* gene and the outcome of

anti-VEGF treatment in AMD. The tSNP rs3025000 is a boundary SNP, located at position 43,746,169 in intron 3 of the *VEGFA* gene, 28 base pairs upstream from the beginning of exon 4 (http://www.ncbi.nlm.nih.gov/sites/nuccore/; accessed April 25, 2011).

Because anti-VEGF medications induce their therapeutic action by blocking all VEGF-A isoforms and VEGF initiates and promotes pathologic CNV, the finding that SNP rs3025000 in the *VEGFA* gene is associated with a significantly better outcome for anti-VEGF treatment in patients with neovascular AMD suggests a potential pharmacogenetic relationship.

A significant association has previously been reported between a haplotype in the *VEGFA* gene including the SNPs rs1413711, rs833070, and rs3025000 and acute mountain sickness (AMS). In that study, the patients with AMS had significantly lower peripheral oxygen saturation levels at high altitude compared with the control group.[20] The mechanism of development of AMS is not fully understood; however, hypoxemia secondary to hypoxia and subsequent vascular changes in the body are the main pathophysiological changes seen in AMS.[21] Another study showed that patients with AMS had significantly higher free plasma VEGF-A levels at high altitude compared with healthy individuals, suggesting a role for increased free plasma VEGF-A levels in the pathophysiology of AMS.[22] It is also known that hypoxia is a major inducer of *VEGFA* gene transcription.[13] The association of the genetic variant rs3025000 with both AMS and the response to anti-VEGF treatment in neovascular AMD is interesting. One possible mechanism of how SNP rs3025000 may increase the risk of AMS is a hypoxia-related pathway, which could have some bearing on VEGF-A levels or function and therefore the efficacy of its antagonists in the treatment of neovascular AMD.

The strengths of our study include the following. This was a single-center study; all eligible patients who underwent anti-VEGF treatment for untreated neovascular AMD at the medical retina clinic of the RVEEH from 2006 to 2010 were included. The mean change in VA of our patients was $+6.5 \pm 12$, $+4.4 \pm 13.4$, and $+2.3 \pm 14.6$ letters after 3, 6, and 12 months, respectively, and compared well with the VA outcomes in the RCTs with similar number of injections over 12 months[5,6]; the distribution of the lesion types (24% predominantly classic versus 76% non-predominantly classic), mean age of patients, and VA response patterns were similar to those of the RCTs.[5,6] In addition, minor allele frequencies for the 7 tSNPs in our cohort were very similar to those in a CEU (Utah residents with ancestry from northern and western Europe) population (Table 4).

The weakness of this study was that the patients were treated with a pro re nata (according to need) regimen. As a result, the patients did not receive exactly the same number of injections over the 12-month course of the study. However, all patients did receive 3 initial monthly injections and the treatment in the first 6 months was very similar for all patients (4–6 injections for most patients). This indicates that the significant association observed between SNP rs3025000 and VA outcome at 6 months anti-VEGF treatment was minimally, if at all, affected by the flexible treatment regimen and actual number of injections. This further strengthens the idea that the 6-month VA outcome is less affected by individual treatment regimens and would be a good time point to look for the influence of genetic variation on the outcome. The outcome at around 6 months after the treatment commencement is in the so-called critical predictive window whereby the course a patient's vision is going to take over the next 12 to 24 months is determined. Over the first 6 months of treatment, patients with the presence of the minor T allele (TT or TC genotypes) at SNP rs3025000 received a mean ± SD of 4.1 ± 1.1 injections compared with 4.1 ± 1.2 injections in patients without the minor T allele (CC genotype) at this SNP. This indicates that the number of injections was unlikely to be a confounding factor for the significant association of genetic variants at SNP rs3025000 and the outcome of treatment at 6 months.

The decision to re-treat in the flexible phase was solely at the discretion of the retinal specialists on the basis of the re-treatment criteria at each visit. They were totally masked to the genotype of the patient so that treatment decisions should have been random in relation to the SNP profile. The drop in mean improvement in VA after 6 months may indicate the injection frequency after 6 months was less than optimal.

Patients with the presence of 1 or 2 copies of the minor allele at SNP rs3025000 showed similar visual outcomes to those of the ANCHOR and MARINA RCTs. This was achieved with 6.3 ± 2.1 injections over 12 months compared with monthly injections in those RCTs. On the other hand, the visual outcome of the patients with the CC genotype at this SNP, despite a similar number of injections (6.2 ± 2.5 injections over 12 months), never rose to the level seen with the TT or TC genotypes and was poorer than those of the weighted ANCHOR/MARINA over the pro re nata period (Figure 1). This suggests that the patients with the CC genotype at SNP rs3025000 may require more frequent injections to achieve the optimal early outcomes or may lack the ability to respond as well as the others from the treatment onset. The proportion of different lesion types in patients with TT or TC genotypes was similar to that in patients with CC genotype at SNP rs3025000. In addition, in a preliminary analysis of OCT characteristics after 3 injections, we could not link the VA outcomes in the various genotypic groups with the presence or absence of fluid on OCT image analysis (unpublished data).

The evidence provided suggests that there is a pharmacogenetic interaction between the anti-VEGF treatment and VA outcomes. Genetic variants within the *VEGFA* gene seem to play an important role in treatment outcome after anti-VEGF treatment. More individualized treatment regimens, based on the *VEGFA* genotype variants, may offer optimal visual outcomes in patients receiving anti-VEGF treatment for neovascular AMD. Patients with the TC or TT genotypes at SNP rs3025000 may achieve promising outcomes with fewer injections, which makes the treatment more cost effective. Further validation studies will lead to a better understanding of determinants of VA outcome with this common treatment for neovascular AMD.

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Variants in the *APOE* Gene Are Associated with Improved Outcome after Anti-VEGF Treatment for Neovascular AMD

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Purpose. Anti-vascular endothelial growth factor (anti-VEGF) drugs have dramatically improved the treatment of neovascular AMD. In pivotal studies, almost 90% of patients maintain vision, with approximately 30% showing significant improvement. Despite these successes, 10% to 15% of patients continue to lose vision, even with treatment. It has been reported that variants in some AMD-associated genes influence treatment outcome. This study showed an association of treatment outcome with variants in the apolipoprotein E (*APOE*) gene.

Methods. One hundred ninety-two patients receiving anti-VEGF treatment for subfoveal choroidal neovascularization secondary to AMD were enrolled. Information on demographics, lesion characteristics, delay until treatment, visual acuity (VA), and number of treatments was collected, and variants of APOE were assessed in all patients at baseline. Best corrected logarithm of the minimum angle of resolution (logMAR) VA was recorded in all patients.

Results. The presence of the *APOE* ε4 allele was associated with improved treatment outcome at 3 ($P = 0.02$) and 12 ($P = 0.06$) months, compared with the presence of the ε2 allele, after adjustment for baseline acuity, treatment delay after first symptoms, age, and sex. Patients with an APOE ε4 allele had an odds ratio (OR) of 4.04 (95% confidence interval [CI], 1.11–14.70) for a 2-line gain in vision from baseline at 3 months ($P = 0.03$) and an OR of 2.54 (95% CI, 0.61–10.52; $P = 0.20$) at 12 months after treatment, based on multivariate analysis.

Conclusions. In patients with neovascular AMD, the presence of the *APOE* ε4 allele conferred significantly better visual outcomes after anti-VEGF treatment than did the ε2 allele. These findings suggest a possible role for a personalized approach to treatment with anti-VEGF. (*Invest Ophthalmol Vis Sci.* 2011;52:4072–4079) DOI:10.1167/iovs.10-6550

Age-related macular degeneration (AMD) is the most common cause of severe, irreversible loss of vision among elderly populations in the developed world.[1–4] The neovascular, or exudative, form of the disease destroys central vision rapidly and is responsible for most severe vision loss in AMD. Ranibizumab is a recombinant, humanized, monoclonal antibody that neutralizes all isoforms of vascular endothelial growth factor (VEGF), a key mediator of the neovascular process.[5–9] The MARINA[10] and ANCHOR[11] studies demonstrated that ranibizumab was an effective treatment for neovascular disease. Vision remained stable in approximately 90% and improved in approximately one third of treated patients. Published case series of bevacizumab treatment showed similar outcomes.[12,13] Unfortunately, approximately 10% of treated patients[10,11] continued to lose vision. Trials assessing the efficacy of variable dosage regimens have generally shown a less favorable vision improvement, compared to a monthly regimen. The PIER trial[14] used a quarterly regimen of ranibizumab after three loading-dose injections. Although the treated group had better vision at 12 months, compared with the sham-treated patients, the initial gains in visual acuity observed over the loading-dose period were lost, and the vision at 12 months was similar to baseline. It was noted that not all patients treated with this regimen fared equally/ Some patients maintained vision gains that were achieved during the monthly-dose period and others either did not respond at all or initially responded well and then failed to maintain the initial gains. The SUSTAIN trial (Meyer CH, et al. *IOVS* 2008;49:ARVO E-Abstract 273) provided further evidence of this differential response to treatment. In this trial, ranibizumab treatment was examined on a pro re nata basis, after administration of three initial loading-dose injections, one in each of three consecutive months. Retreatment was based on specific visual acuity and clinical and optical coherence tomography criteria. Of these patients, approximately 50% maintained their initial sharp gains in visual acuity, the so-called gain-and-maintain group. Another third of patients enjoyed an initial gain in visual acuity, but failed to maintain the gain during follow-up: the gain-and-not-maintained group. Most of the acuity increase, in these two groups, occurred in the first 3 months of treatment. Approximately 25% of patients never experienced any gain in vision: the no-initial-gain group. In these patients, vision failed to improve from the outset, with continued losses being noted during the course of the treatment regimen. Overall, approximately 20% of the patients maintained their visual acuity without any further treatment after the initial loading doses.

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Currently, we do not fully understand what factors dictate this response. If we did, we might be able to predict patient outcomes after anti-VEGF treatment more accurately. Recent studies, including the MARINA and ANCHOR subgroup analyses, suggest that baseline visual acuity, lesion size, and age influence outcomes of anti-VEGF therapy.[15–17] The potential role of genetic variation in determining outcome has also been investigated. Although multiple studies have implicated roles for several genes, including *CFH, HTRA1/ARMS2, C3, CFB/C2,* and *APOE,* in the risk of developing AMD, there is no consensus in the literature regarding the influence of these genetic polymorphisms in the response to anti-VEGF treatment.[18–23] The *CFH* gene has been the most widely investigated, with conflicting reports that the CFH at risk allele CC is associated with favorable or adverse treatment responses.[18–23] No specific associations with polymorphisms of the *C3* or *ARMS2/HTRA1* genes in determining treatment outcome have been noted, although it has been suggested that polymorphisms within the C-reactive protein (*CRP*) gene are associated with worse visual outcome after anti-VEGF treatment.[20] However, these studies were limited by small patient samples that left them underpowered, and they did not account for all other potentially confounding factors that can affect visual outcome.

Nongenetic variables that have been shown to influence visual outcome and should be included in any attempt to identify genetic influences on outcomes include age, presenting visual acuity, and the size and type of lesion.[10,11,24] Traditionally, the time delay between the first symptoms of neovascular AMD and initiation of treatment was an important determinant of outcome, when the treatment was thermal laser photocoagulation, which could be applied successfully only if the choroidal neovascularization (CNV) was treated before it became subfoveal. Given that these new medical treatments are used in subfoveal lesions, it is not necessarily a given that delay to treatment would influence outcomes. It has been reported that this variable remains an important parameter in response to anti-VEGF therapy,[25,26] although it is difficult to assess accurately and thus is rarely ascertained. We have found in our present cohort of patients that delay of treatment after the first symptoms suggestive of CNV is a highly significant predictor of outcome (Lim et al., manuscript submitted). Lack of consideration of this variable may well explain the contradictory results of previously published studies in which treatment outcomes with anti-VEGF were investigated. Thus, we sought to address the possible genetic variants and their influence on treatment outcome by conducting a study that took into consideration variables that we found to have a significant effect on outcomes.

In this study we chose to look at the potential influence of polymorphisms of the *APOE* gene in determining outcome. We and others[27–31] have demonstrated a higher likelihood of development of AMD in individuals with the *APOE* ε2 polymorphism than with the ε3 and ε4 variants. As such, we were interested in determining whether there is any genetic influence on treatment outcome, within the variants of the *APOE* gene. We examined visual acuity outcome at 3, 6, and 12 months after initiation of treatment. Several pivotal RCTs,[10,11,14] have clearly demonstrated that in most cases, visual acuity increases occurred in the first 3 months of treatment. Also in those patients who responded poorly, vision failed to improve from the outset, with continued losses noted early during treatment. Investigating VA outcomes at 3 months therefore accounted for a very uniform phase in treatment strategy. At 6 and 12 months, other variables such as injection timing and number as well as missed appointments may play a role. Thus, we felt it useful to investigate an early time point as well as later ones during the course of treatment to see the extent of possible genetic influence on outcome.

Methods

Study Design and Eligibility

This study was approved by the Human Research and Ethics Committee of the Royal Victorian Eye and Ear Hospital (RVEEH), as part of the Age-Related Macular Degeneration Inheritance Study. Research adhered to the tenets of the Declaration of Helsinki, and the participants provided written informed consent before participation.

Our study population consisted of patients from the Medical Retina Clinic at the RVEEH and the private rooms of the Eastern Retinal Service (Box Hill, Melbourne).

Exclusion criteria were (1) CNV secondary to other, non-AMD conditions such as angioid streaks, degenerative myopia, central serous retinopathy or hereditary retinal disorders; (2) laser photocoagulation or photodynamic therapy before anti-VEGF administration; and (3) non-Caucasian ancestry.

Data Collection and Follow-up

Data pertaining to patient demographics, medical history, visual acuity (VA), and treatment scheduling were collected after consent was obtained. A peripheral blood sample was also collected for DNA extraction.

All patients underwent a dilated fundus examination. Snellen VA was recorded by clinic orthoptists who were masked to the study and used a standard chart at a standard distance of 6 m to get best corrected VA. At each appointment, VA was reassessed in the same, standardized manner, albeit by different orthoptists in many cases. These values were converted to logarithm of the minimum angle of resolution (logMAR) values for all study calculations and comparisons. Fluorescein angiography was performed by the Medical Photographic Imaging Centre at the RVEEH or by an orthoptist at the private Eastern Retinal Service as part of normal patient management. Angiograms were accessed via a digital imaging system (IMAGEnet 2000; Topcon Corp., Tokyo, Japan). A retrospective review of all angiograms was undertaken, and lesion size was measured in comparison to optic disc area using the middle phase frame of the angiogram.

Treatment Decisions

All treatment decisions were based solely on the discretion of the treating retinal specialist. No specific retreatment strategies were implemented, although 75% of the participants initially received one injection during each of three consecutive months. The treatment used by the participating physicians most closely resembled an as-needed strategy, although if extension between treatments was considered, the clinical decision in general was to treat without extending treatment intervals until the retina was free of fluid. Both ranibizumab and bevacizumab were used during the treatment period, and in some cases, both drugs were used in the same eye at different time points.

Genotyping

The single-nucleotide polymorphisms (SNPs) rs429358 and rs7412 of the *APOE* gene were genotyped as previously reported.[29] In brief, the polymerase chain reaction (PCR) amplification was undertaken with the primers AF (5′GCCTCCCCACTGTGCGA3′), AR (5′GGCCGAGCATGGCCTG3′) for rs429358 and BF (5′ACCGAGGAGCTGCGGG3′), BR (5′CTCGCGGATGGCGCTGA3′) for rs7412. Digestion of each PCR product was undertaken with the restriction enzymes *Afl*III for rs429358 and *Hae*II for rs7412. Digested products were size separated on agarose gels. The combination of amino acids at these two SNPs allowed the common allelic variants of ε2, ε3, or ε4 of the *APOE* gene to be determined. Dideoxynucleotide sequencing was undertaken on a representative sample to confirm the results of each restriction digestion profile.

Statistical Analysis

The primary measure of this study was VA outcome after anti-VEGF treatment for neovascular AMD. Improvement in VA was defined as a 2-line gain in VA, as measured on a Snellen chart at 3 and 6 months after the first treatment. Stable vision was defined as VA within 2 lines of baseline after the same time period. A decrease was considered to be a reduction in VA of 2 lines or more.

In addition to patient genotype, other possible predictors of eventual outcome were investigated and defined based on the following characteristics: (1) age (years); (2) sex; (3) smoking status (nonsmoker, past or current smoker); (4) delay between first symptoms of CNV and first treatment; (5) delay between confirmed CNV diagnosis and first treatment; (6) lesion type (predominantly classic, nonpredominantly classic); (7) lesion size (≤2 disc areas [DA], >2 DA); (8) number of treatments (injections) given in the 6-month follow-up period; injection number (≤3, >3]); and (9) baseline VA.

All these treatment subgroups were selected before the study commenced.

When both eyes of a patient were recruited into the study, the eye with worse vision at the end of the 12-month follow-up period was selected for analysis. Data are reported as the median and interquartile range (IQR; p25, p75 in the tables) or the mean ± standard deviation (SD) for continuous variables, and as proportions for categorical variables. For the categorical variables, χ^2 analysis was used to determine whether there were any significant differences in the treatment outcome between each of the subgroups. For the continuous variables, ordinal logistic regression analysis was used.

To determine the extent of the effect of the specific SNPs of the *APOE* gene and their relationship with treatment outcomes, we used univariate logistic regression to generate odds ratios (ORs) for effect of genotype. To assess the effect of potentially confounding variables other than patient genotype, univariate logistic regression analysis was also used. All factors with a univariate significance level of $P < 0.10$, as well as for age and sex, were included in the multivariate ordinal regression model to see whether any significant effects of genotype in the univariate analysis remained (Stata Statistical Software Release 10.0; Stata Corp., College Station, TX).

Sample Size

Assuming an effect of the *APOE* polymorphisms on vision outcome with treatment, we calculated the sample size to allow for a 25% increased chance for vision improvement with ε4 variant compared with the ε2 variant. To detect a significant difference ($P = 0.05$) between polymorphisms of the *APOE* gene, we calculated that we would require a minimum sample size of 152 eyes for the desired statistical power of 80%.

Results

Population Characteristics

Of the 198 eyes of 192 patients that were enrolled, 30 eyes were excluded from the analysis for the following reasons: The *APOE* status of 13 patients (13 eyes) could not be determined (failed genotyping); 4 patients, with both a risk and protective allele, had a potentially confounding ε2/ε4 genotype; and 6 eyes of 6 patients received bilateral anti-VEGF treatment for CNV, and of those, only the eye with the worse outcome at 12 months was considered for analysis. Finally, seven patients contributing seven eyes failed to complete follow-up. These exclusions resulted in a final study population of 168 patients (168 eyes), of whom the majority (98; 58.3%) were women. Most lesions (122; 72.6%) were classified as nonpredominantly classic (occult and minimally classic), and most lesions (103; 66.0%) were less than or equal to 2 DA in size (103; 66.0%). The median time delay from detection of the first symptoms suggestive of CNV until treatment was 12 weeks, and at the baseline visit, the mean logMAR VA was 0.60.

Of the 168 eyes, 101 had only ranibizumab treatment, and 67 had a combination of ranibizumab and bevacizumab. No differences were observed in visual outcome between the two groups with respect to frequency of the *APOE* alleles ($P = 0.92$) and treatment outcome ($P = 0.27$). Consequently, the patients were combined and analyzed as a single cohort. Table 1 summarizes the patient and eye characteristics that we analyzed, by treatment outcome at 3, 6, and 12 months. The responses were classified as improved, stable, or decreased VA. At 3 months, in 28.3% VA had improved by 2 or more lines, in 64.5% it was stable, and in 7.2% it had worsened by 2 or more lines. Similarly, at 6 and 12 months VA in 29.2% and 24.2%, respectively, had improved, whereas in 57.7% and 57.1% it was stable; in 13.1% and 18.7% at 6 and 12 months, respectively, it had deteriorated. Of the study participants, the median age was 80 years (range, 62–94 years). There appeared to be a difference in the outcome of younger patients compared with older patients at 3, 6, and 12 months, with the median age of those improving vision at all three time periods being 78, 77, and 79 years, respectively, compared with 81 years at all three time points in those with stable vision or loss of vision. There was no significant difference in distribution across the treatment outcome categories with respect to sex or smoking status, although there was a trend for women and nonsmokers to fare better than men and previous or present smokers.

The putative AMD-risk ε2 allele for the *APOE* gene was identified in 17.4% of all patients, whereas the protective e4 allele was present in 16.1% of patients. At 3 months, the presence of at least one ε2 allele (ε2 /ε2 or ε2/ε3) was associated with a 2-line improvement in VA in only 16.7% of carriers, whereas 45.5% of ε4 (ε4 /ε4 or ε4 3/ε4) carriers showed this improvement. In patients with only the ε3 allele (ε3/ε3), 27.5% improved vision by two or more lines, $P = 0.06$. Looking specifically at the presence of the ε2 allele compared with the presence of the ε4 allele, we found a significant difference in outcome. The presence of the ε4 allele was associated with a greater likelihood of vision improvement ($P = 0.02$) at 3 months. A similar, but not significant, trend was noted at 6 and 12 months, with a greater percentage of patients with the ε4 allele compared with the ε2 allele showing vision improvement (33.3% vs. 12.3%, $P = 0.07$ and 37.5% vs. 15.4%, $P = 0.06$) at 6 and 12 months, respectively.

Contrary to the *APOE* ε4 findings, we did not detect any significant difference at 3, 6, or 12 months with regard to whether the *APOE* ε2 allele conferred a poorer outcome through loss of 2 lines or greater in vision. At 3 months 2 (6.7%) of 30 eyes with ε2 deteriorated by 2 lines or more compared with 3 (11.1%) of 27 eyes with ε4 ($P = 0.50$). Similarly at 6 months, 3 (10.0%) of 30 eyes with ε2 deteriorated compared with 4 of (14.8%) 27 eyes with ε4 ($P = 0.58$). Of note, at 12 months, although not significant, 6 (23.1%) of 26 eyes with ε2 deteriorated compared with 2 (8.3%) of 24 of those with ε4 ($P = 0.155$).

Lesion type, the number of injections, and lesion size failed to show any relationship to vision outcome, although there was borderline significance for lesion size at 3 months ($P = 0.07$), with lesions 2 DA or smaller more likely to be associated with improved vision. Also, at 12 months, predominantly classic lesions tended to have better visual outcome (35% vs. 20%, $P = 0.07$). Baseline VA was a highly significant determinant of response, with better presenting VA more likely to be associated with a deterioration in vision compared with worse vision at baseline. We hypothesize, as have others, that this association is related to a ceiling effect

TABLE 1. Patient and Study Eye Characteristics by Treatment Outcome at 3, 6, and 12 Months

Characteristics	3-mo Outcome (n = 166)			6-mo outcome (n = 168)			12-mo outcome (n = 149)		
	Stable/Worse VA	Improved VA	*P*	Stable/Worse VA	Improved VA	*P*	Stable/Worse VA	Improved VA	*P*
Age, median (p25, p75)	81 (76, 85)	78 (73, 82)	0.16	81 (77, 85)	77 (72, 82)	0.03	81 (77, 85)	79 (73, 81)	0.03
Sex									
Male	45 (64)	25 (36)	0.07	44 (37)	26 (53)	0.06	41 (36)	18 (50)	0.14
Female	74 (77)	22 (23)		75 (63)	23 (47)		72 (64)	18 (50)	
Smoking									
Nonsmoker	49 (79)	13 (21)	0.09	47 (43)	16 (35)	0.36	41 (38)	16 (47)	0.37
Past/present smoker	61 (66)	31 (34)		63 (57)	30 (65)		66 (62)	18 (53)	
APOE genotypes									
ε2/ε2; ε2/ε3	25 (83)	5 (17)	0.06	26 (22)	4 (8)	0.11	22 (19)	4 (11)	0.25
ε3/ε3	79 (72)	30 (28)		75 (63)	36 (74)		76 (67)	23 (64)	
ε3/ε4; ε4/ε4	15 (55)	12 (45)		18 (15)	9 (18)		15 (13)	9 (25)	
APOE alleles (ε2 vs. ε4)									
ε2	25 (83)	5 (17)	0.02	26 (87)	4 (13)	0.07	22 (85)	4 (15)	0.06
ε4	15 (55)	12 (45)		18 (67)	9 (33)		15 (62)	9 (38)	
Baseline logMAR VA median (p25, p75)*	0.60 (0.40, 0.90)	0.70 (0.48, 1.00)		0.60 (0.40, 0.78)	0.78 (0.48, 1.00)	0.02	0.60 (0.48, 0.78)	0.74 (0.50, 1.00)	0.01
Time delay: symptoms to treatment, wk									
Lowest tertile (<7)	34 (64)	19 (36)	0.22	34 (62)	20 (38)	0.32	34 (76)	11 (24)	0.72
Middle tertile (7–21)	36 (80)	9 (20)		32 (71)	13 (29)		35 (80)	9 (20)	
Highest tertile (>21)	32 (70)	14 (30)		36 (77)	11 (23)		31 (72)	12 (18)	
Time delay: diagnosis of CNV to treatment, wk									
Lowest tertile (<1)	51 (69)	23 (31)	0.21	55 (73)	20 (27)	0.33	50 (78)	14 (22)	0.02
Middle tertile (1–3)	31 (67)	15 (33)		29 (63)	17 (37)		27 (61)	17 (39)	
Highest tertile (>3)	37 (82)	8 (18)		35 (76)	11 (24)		36 (70)	5 (30)	
CNV lesion type									
Predominantly classic	29 (66)	15 (34)	0.30	32 (73)	12 (27)	0.78	26 (65)	14 (35)	0.07
Non-predominantly classic†	89 (74)	31 (16)		86 (70)	36 (30)		86 (80)	22 (20)	
Total CNV lesion size									
≤2 DA	68 (67)	33 (33)	0.07	71 (69)	32 (31)	0.27	67 (74)	24 (26)	0.47
>2 DA	43 (81)	10 (19)		41 (77)	12 (23)		38 (79)	10 (21)	
Number of treatments									
≤3	49	119	—	58 (64)	23 (36)	0.94	33 (85)	6 (15)	0.22
>3	—	—	—	59 (71)	24 (29)		78 (75)	26 (25)	

n (%) for categorical variables; median (p25, p75) for continuous variables. Percentages may not total 100 due to rounding. Apo ε2 is constituted by the ε2/ε2 and ε2/ε3 genotypes; Apo ε4 is constituted by the ε3/ε4 and ε4/ε4 genotypes.

* VA worsens as logMAR value increases.

† Includes minimally classic, occult, retinal angiomatous proliferation, fibrovascular pigment epithelial detachment.

TABLE 2. Univariate Logistic Regression Analysis of Treatment Outcomes

Characteristics	3-mo Outcome (n = 166)		6-mo Outcome (n = 168)		12-mo Outcome (n = 149)	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age, y	0.95 (0.91, 1.00)	0.04	0.94 (0.89, 0.99)	0.02	0.94 (0.89, 0.99)	0.02
Sex						
Male	1	0.07	1	0.06	1	0.15
Female	0.54 (0.27, 1.06)		0.52 (0.26, 1.02)		0.57 (0.27, 1.22)	
Smoking status						
Nonsmoker	1	0.09	1	0.36	1	0.37
Past/present smoker	1.91 (0.90, 4.06)		1.39 (0.68, 2.86)		0.69 (0.32, 1.53)	
Time delay: symptoms to treatment, wk						
Lowest tertile	1		1		1	
Middle tertile	0.44 (0.18, 1.12)	0.09	0.69 (0.29, 1.62)	0.39	0.79 (0.29, 2.16)	0.65
Highest tertile	0.78 (0.34, 1.82)	0.57	0.52 (0.22, 1.25)	0.14	1.19 (0.46, 3.11)	0.71
Baseline logMAR VA*	2.67 (1.31, 5.41)	0.007	3.24 (1.56, 6.74)	0.002	4.45 (1.81, 10.98)	0.001
CNV lesion type						
Predominantly classic	1	0.30	1	0.78	1	0.07
Nonpredominantly classic†	0.67 (0.32, 1.42)		1.12 (0.52, 2.41)		0.47 (0.21, 1.06)	
Total CNV lesion size						
≤2 DA	1	0.07	1		1	0.47
>2 DA	0.48 (0.21, 1.07)		0.64 (0.30, 1.40)	0.27	0.74 (0.32, 1.70)	
Number of treatments						
≥3	—		1	0.94	1	0.23
>3	—		1.03 (0.52, 2.02)		1.83 (0.69, 4.88)	
APOE alleles						
ε2	1		1		1	
ε3	1.89 (0.66, 5.43)	0.23	3.12 (1.01, 9.64)	0.04	1.66 (0.52, 5.34)	0.39
ε4	4.00 (1.17, 13.65)	0.03	3.25 (0.86, 12.24)	0.08	3.30 (0.85, 12.77)	0.08

APOE alleles are as described in Table 1.

* VA worsens as logMAR value increases.

† Includes minimally classic, occult, retinal angiomatous proliferation, fibrovascular pigment epithelial detachment, and fibrosis, hemorrhage.

that prevents people with good presenting vision from showing improvement.

Previously, we have reported a significant association between the outcome of treatment and the length of delay between initial symptoms of CNV and treatment (Lim et al., manuscript submitted), after considering the entire range of responses to treatment (improved, stable, or worse vision). For the current analysis, we sought to define characteristics that would predict a better vision outcome, and, as such, we grouped the eyes with stable and worse vision together. In this analysis, no significance was found for time until treatment in predicting response. This result suggests that longer delays are associated with worse response, rather than shorter delays being associated with a better response.

Univariate Analysis

Table 2 shows the univariate ordinal logistic regression models for the *APOE* gene, as well as other potential contributory factors such as age, sex, size of CNV membrane, and delay from initial symptoms to initiation of treatment.

A statistically significant relationship ($P = 0.04$) was identified between the ε4 polymorphism and better posttreatment VA outcomes. The presence of the ε4 allele conferred an increased chance of better than 2 lines of vision improvement with treatment (OR, 4.00; 95% confidence interval [CI], 1.17–13.65, at 3 months, $P = 0.03$). At 6 and 12 months, a similar trend was present, although not statistically significant (OR, 3.25; 95% CI, 0.86–12.24; $P = 0.08$; and OR, 3.30; 95% CI, 0.85–12.77; $P = 0.08$, respectively). Of all other variables analyzed, baseline VA also correlated significantly with vision at 3, 6, and 12 months, with eyes that had worse initial VA more likely to have a 2-line vision improvement (OR, 2.67; 95% CI, 1.31–5.41; $P = 0.007$; OR, 3.24; 95% CI, 1.56–6.74; $P = 0.002$; and OR, 4.45; 95% CI, 1.81–10.98; $P = 0.001$, respectively).

Multivariate Analysis

Table 3 shows the multivariate regression model for the *APOE* gene. All patient or clinical characteristics with a significance level of $P < 0.10$ in the univariate analysis were incorporated into a final analysis. Younger patients tended to have an increased likelihood of vision improvement compared to older patients at 6 and 12 months (OR, 0.94; 95% CI, 0.89–0.99; $P = 0.03$ and OR, 0.94; 95% CI, 0.88–0.99; $P = 0.04$, respectively). No significant difference was seen at 3 months. Better initial acuity remained a significant predictor of a poorer response to treatment when adjusting for age, baseline VA, and genotype.

The presence of the *APOE* ε4 allele was a statistically significant predictive factor for better treatment outcome at 3 months (OR, 4.04; 95% CI, 1.11–14.70; $P = 0.03$). At 6 months (OR, 3.26; 95% CI, 0.76–13.90; $P = 0.11$) and 12 months (OR, 2.54; 95% CI, 0.61–10.52; $P = 0.20$), similar, but nonsignificant trends were observed.

DISCUSSION

Anti-VEGF drugs have revolutionized the treatment and visual outcome of patients with neovascular AMD. These treatments offer, for the first time, a real possibility of improving vision. However, vision does not improve in all treated patients; approximately 10% of patients continue to lose vision despite treatment. It is not known why some individuals do not benefit from treatment with these anti-VEGF agents, but genetic make-up may be a determinant. In this study we investigated the role of the *APOE* gene and its relation to treatment outcome in patients with neovascular

TABLE 3. Multivariate Logistic Regression Analysis of Treatment Outcomes

	3-mo Outcome (n = 166)		6-mo Outcome (n = 168)		12-mo Outcome (n = 149)	
Characteristics	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Age, y	0.96 (0.91, 1.01)	0.18	0.94 (0.89, 0.99)	0.03	0.94 (0.88, 0.99)	0.04
Sex						
Male	1	0.20	1	0.16	1	0.44
Female	0.62 (0.30, 1.28)		0.60 (0.30, 1.22)		0.73 (0.33, 1.61)	
Baseline logMAR VA*	2.80 (1.31, 6.04)	0.001	4.18 (1.87, 9.33)	0.001	4.88 (1.89, 12.58)	0.001
APOE allele						
ε2	1		1		1	
ε3	2.27 (0.77, 6.69)	0.14	4.32 (1.24, 15.06)	0.02	1.93 (0.61, 6.11)	0.26
ε4	4.04 (1.11, 14.70)	0.03	3.26 (0.76, 13.90)	0.11	2.54 (0.61, 10.52)	0.20

Each OR and *P* value is adjusted for all other characteristics listed in the table. *APOE* alleles are as described in Table 1.

* VA worsens as logMAR value increases.

AMD. In an approach that has never been undertaken, we attempted to account for all other important confounding factors by adjusting for patient and lesion characteristics as well as treatment delay.

We showed that *APOE* genotype is significantly associated with treatment outcome. The presence of an *APOE* ε4 allele, significantly increased the chances of a 2-line improvement in VA at 3 months, with borderline significance at both 6 and 12 months.

We also demonstrated that presenting VA and age of the patient were other factors associated with visual outcome.

APOE may play a role in several pathologic processes thought to be occurring in AMD. *APOE* is expressed in photoreceptor outer segments, the retinal ganglion layer and in Bruch's membrane. It is also secreted by RPE cells.[32,33] It has been postulated that *APOE* ε4 allele may facilitate greater lipid efflux from RPE cells and lipid transport across Bruch's membrane,[30] an effect secondary to the prevention of normal dimerization of the apolipoprotein E protein that usually occurs with the *APOE* ε3 and ε2 isoforms.

People with one or two copies of *APOE* ε4 have reduced C-reactive protein (CRP) levels when compared to individuals lacking these copies.[34,35] CRP is a marker of systemic inflammation and is a prominent drusen-associated molecule. It is expressed in significantly higher levels among patients with advanced AMD.[36]

In addition, the effects of the *APOE* isoforms on chemokine and cytokine regulation may offer some insight into the mechanism responsible for the observed protective role of the ε4 isoform. Oxidative stress has been implicated in the development of AMD,[37,38] under such conditions, increased levels of both CCL2 (macrophage chemotactic protein) and VEGF have been demonstrated.[39,40] Both VEGF and CCL2 overexpression have been associated with further degeneration, especially in advanced disease stages of AMD.[41] Bojanowski et al.[42] demonstrated an almost twofold suppression of both CCL2 and VEGF expression in RPE cells by the *APOE* ε4 isoform compared to ε3 and ε2.

The *APOE* ε4 isoform may therefore have a protective role in preventing AMD development and progression as well as allowing a better response to VEGF inhibition.

Our results suggest a significant effect of *APOE* ε4 and outcome at 3 months, with borderline significance thereafter. The loss of significance with longer time of follow-up may well be because other variables, such as treatment interval or missed appointments, should be factored into these longer time period analyses. Notably, at 3 months, most patients (75%) had received three monthly loading-dose injections, whereas after this time, clinicians treated on their own discretion, with the result that a variable number of treatments were given at different intervals, depending on the treating ophthalmologist.

We did not find any significant deleterious effects of the *APOE* ε2 allele on visual outcomes; however, in keeping with the suggestion that the *APOE* gene may influence treatment outcome, Lee et al.[43] found that, in the eyes of transgenic mice expressing human *APOE* ε2, VEGF was overexpressed in the RPE. This works suggests that this risk allele alters or upregulates angiogenic cytokines and influences the amount of VEGF in the retinas of these patients.. Indeed, in our previous study of *APOE* and AMD, we found that individuals with the ε2ε3 genotype had a significantly younger mean age of disease diagnosis, particularly with choroidal neovascular disease, compared with patients with the ε3ε3 genotype (4.7 years, $P = 0.003$).[29]

Our finding that the presence of the *APOE* ε4 allele is associated with an increased chance of better anti-VEGF treatment outcome, at least in the early stage of treatment, has important research and clinical implications. As there are possibly lower retinal VEGF levels in these patients, it may well be that less intensive dosage regimens of anti-VEGF would be sufficient to counteract the relatively lower level of VEGF present. In addition, knowing about the presence of a particular *APOE* allele will allow better prognostic information for individuals at the start of treatment, thus ushering in the start of personalized anti-VEGF treatment in neovascular AMD. Further prospective studies with larger cohorts should be conducted to validate our study results. New genetic studies are needed, to investigate the associations of anti-VEGF treatment outcomes with other genes, including the VEGF-A gene itself, as its product is the key molecule promoting abnormal blood vessel growth and is the target of therapy.

The design of our study is a major strength. We investigated and adjusted for patient and lesion characteristics, treatment delay, as well as genetic polymorphisms, all in the same cohort of patients. The observational nature of our study allowed accurate representation of normal clinic patients and normal treatment procedures, as opposed to a randomized clinical trial, best enabling retinal specialists to apply our findings to the clinical environment. Our study population was also larger than any comparable clinical study of genetic determinants of treatment outcomes. Potential limitations should also be mentioned. A larger sample size may have allowed us to find significant differences in outcomes with different genotypes when we found a trend at 6 and 12

months. Second, there was the potential for selection bias, as it was not possible to recruit every patient, such as those who required an interpreter (for non–English-speaking patients of European origin). This lowered the representation of populations groups that were not fluent in English (e.g., Mediterranean and East European persons).

In summary, we evaluated potential predictors of anti-VEGF treatment outcomes in neovascular AMD, to maximize the benefit of these therapies and to minimize harm to those who are unlikely to gain from ongoing treatment. In this study, the presence of the *APOE ε4* allele was significantly associated with a better chance of significant vision improvement in patients treated with anti-VEGF agents for neovascular AMD. Given the substantial number of people with AMD, further investigation into this potential pharmacogenetic relationship is needed to guide the use of these revolutionary treatments.

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Retinal Venular Caliber Predicts Visual Outcome after Intravitreal Ranibizumab Injection Treatments for Neovascular AMD

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Purpose. To examine whether baseline retinal vascular caliber predicts visual response to intravitreal ranibizumab injections in patients with neovascular age-related macular degeneration (AMD).

Methods. In this prospective cohort study, patients with neovascular AMD received three monthly intravitreal injections of ranibizumab, followed by as needed dosing up to 1 year. Retinal vascular caliber was measured from digital fundus photographs at baseline and summarized as central retinal artery equivalent (CRAE) and venular equivalent (CRVE), representing average caliber of arterioles and venules, respectively. Visual outcome at 12 months was assessed and the relation to baseline retinal vascular caliber was determined.

Results. A total of 88 eyes were analyzed at baseline. After accounting for age, sex, size of choroidal neovascularization, and number of injections, patients who deteriorated in visual acuity at 12 months had significantly larger baseline CRVE, 243.10 μm (95% confidence interval [CI], 227.01–259.19), compared with those who were stable, 214.30 μm (95% CI, 205.79–222.81) and those who improved, 215.26 μm (95% CI, 204.69–225.84; $P = 0.007$). Baseline CRAE did not differ significantly from eyes whose vision deteriorated, 150.12 μm (95% CI, 140.67–159.57), compared with those remaining stable, 143.64 μm (95% CI, 138.64–148.63), or gaining vision 142.92 μm (95% CI, 136.71–149.13; $P = 0.69$).

Conclusions. In eyes with neovascular AMD treated with intravitreal ranibizumab, larger baseline retinal venular caliber was significantly associated with a poorer response to treatment, possibly reflecting increased disease severity. (*Invest Ophthalmol Vis Sci.* 2012;53:37–41) DOI:10.1167/iovs.11-7689

Antivascular endothelial growth factor (anti-VEGF) agents have significantly improved the treatment of individuals with neovascular age-related macular degeneration (AMD).[1,2] In neovascular AMD, choroidal neovascularization (CNV) has been associated with ischemic changes in the choroid, with increasing levels of ischemia correlating with severity of AMD,[3,4] as well as an overexpression of vascular endothelial growth factor VEGF.[5]

Ranibizumab (Lucentis; Genentech, Inc., San Francisco, CA) is a recombinant, humanized, monoclonal antibody that neutralizes all isoforms of vascular endothelial growth factor-A, a key mediator of the neovascular process.[3–7] The ANCHOR[1] and MARINA[2] studies demonstrated that ranibizumab was an effective treatment for neovascular AMD, and vision remained stable in approximately 90% and improved in approximately one third of treated patients. However, approximately 10% of treated patients continued to lose vision.[1,2] Furthermore, trials assessing the efficacy of variable dosing regimens have generally shown a less favorable response, in terms of vision improvement compared with a monthly dosing regimen.[8,9]

Currently, it is unclear what factors predict response to treatment and identifying predictive factors would allow more precise methods to monitor, treat, and provide prognostic information to patients needing anti-VEGF treatment. Retinal vascular caliber is an indirect indicator of ocular blood flow that can be measured from fundus photographs.[10] Changes in retinal vascular caliber have been linked with systemic and ocular diseases, and may reflect changes in retinal blood flow, inflammation, ischemia, and endothelial function.[11,12] Thus, measurement of retinal vascular caliber may provide a marker of underlying disease severity in patients with neovascular AMD and may increase our understanding of which factors influence visual outcome in patients needing anti-VEGF treatment.

The aim of this study was to investigate the relationship between baseline retinal vascular caliber and visual response at 12 months, in eyes receiving anti-VEGF treatment by ranibizumab for neovascular AMD.

Methods

Study Population

This study was based on data from a prospective study evaluating the predictors of outcome of intravitreal ranibizumab on patients with CNV secondary to AMD. The study was approved by the research and ethics committee of the Royal Victorian Eye and Ear Hospital and followed the tenets of the Declaration of Helsinki.

Patients were recruited consecutively (January 1, 2008 to December 31, 2008) from the retina clinics of the Royal Victorian Eye and Ear Hospital, a tertiary eye care hospital in Melbourne, Australia. The inclusion criteria for this study were eyes with subfoveal/juxtafoveal CNV secondary to AMD that were deemed suitable for treatment with an anti-VEGF drug. There were no visual acuity exclusions, although

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the optical media needed to be clear enough to obtain sufficient quality photographs in the study eye.

Study Eyes

Over the 12-month recruitment period, all treatment naïve eyes with neovascular AMD were considered for inclusion, unless media opacity

Best corrected logarithm of the minimal angle of resolution (logMAR) visual acuity was performed using ETDRS charts, after formal subjective refraction.

Optical Coherence Tomography Acquisition and Analysis

[redacted]

optical coherence tomography
pment (Stratus OCT 3; Zeiss-
experienced technician, using
allowed qualitative scoring of
al fluid (edema/cysts), subreti-
detachments. A quantitative
(CMT) was also made at each

or mobility issues prevented adequate fundus visualization and in the view of the examiner would prevent adequate fundus photography.

Measurement of Retinal Vascular Caliber

All participants had fundus photography performed using a standardized protocol according to the Multi-Ethnic Study of Atherosclerosis.[12] An optic disc-centered photograph was taken of each eye at baseline and at three monthly intervals in all participants (Early Treatment of Diabetic Retinopathy Study [ETDRS], Field 1).[13] All photographs were taken before any treatment (TRC50 EX fundus camera; Topcon Corp., Tokyo, Japan). At baseline, photographs were taken within 48 hours of the first treatment. For each subsequent visit, photographs were taken before any treatment given, if required.

Retinal vascular caliber was measured by a masked grader, using a computer-based program (IVAN, University of Wisconsin, Madison, WI), according to an established protocol.[11,14] In all cases due to the disc centration of photographs, the macula status was not visible to the grader. For each photograph (Fig. 1), all arterioles and venules coursing through an area one-half to one-disc diameter from the optic disc margin were measured and vessel calibers of the largest six were summarized as the central retinal artery equivalent (CRAE) and the central retinal vein equivalent (CRVE) using formulas developed by Hubbard et al.[14] and later modified by Knudtson et al.[15] These equivalents are projected calibers for the central retinal artery and vein.

At baseline and at all subsequent visits,
(OCT) was performed with OCT equi
Humphrey Inc., San Leandro, CA), by an
the radial lines protocol. The protocol
the images for the presence of intraretin
nal fluid as well as pigment epithelial
assessment of central macular thickness
visit.

Treatment with Ranizibumab

After the initial "loading dose " regimen
on a pro re nata ("as needed") schec

w, OCT findings, and patient symptoms. The retreatment criteria
the appearance of intraretinal or subretinal fluid on OCT or new
al or subretinal bleeding. However, if continued loss of vision was
rved, despite three consecutive monthly treatments and clinical
res suggested irreversible retinal damage (fibrosis/atrophy), the
ion to withhold treatment could be made by the treating special-
lthough monthly assessment was continued up to 12 months.
he procedure for injection followed a standard protocol as previ-
described.[16,17] In brief, eyes were prepared with several drops of
hocaine 1% and two drops of 5% povidone–iodine. A lid speculum
used in all cases. Lignocaine (2%) was then administered subcon-
ivally with a 30-gauge needle to the site of the intravitreal injec-

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measurements for their
oefficients for CRAE and

tion. Using a 30-gauge needle, 0.05 mL of ranibizumab (0.5 mg; Novartis Pharmaceuticals, Sydney, Australia) was injected through the pars plana into the vitreous cavity. Chloramphenicol ointment was instilled at the end of the procedure and chloramphenicol drops were given to take home for 3 days.

Intragrader reproducibility of retinal vascular study was excellent (intraclass correlation c CRVE were both >0.98).[15]

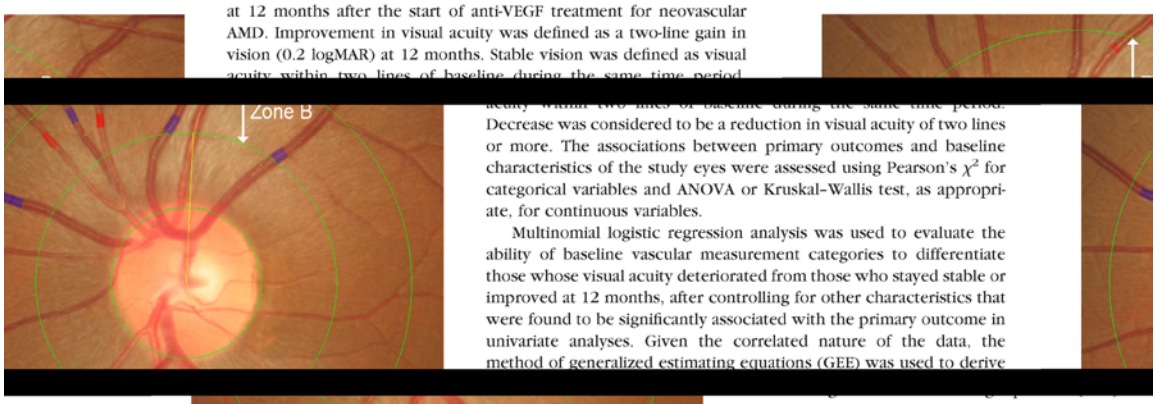
Visual Acuity

Visual acuity was measured at baseline and at masked to vessel measurements and to the

12 months by examiners
treatments administered.

Statistical Methods

The primary outcome measure in this study was change in visual acuity at 12 months after the start of anti-VEGF treatment for neovascular AMD. Improvement in visual acuity was defined as a two-line gain in vision (0.2 logMAR) at 12 months. Stable vision was defined as visual acuity within two lines of baseline during the same time period.



Decrease was considered to be a reduction in visual acuity of two lines or more. The associations between primary outcomes and baseline characteristics of the study eyes were assessed using Pearson's χ^2 for categorical variables and ANOVA or Kruskal–Wallis test, as appropriate, for continuous variables.

Multinomial logistic regression analysis was used to evaluate the ability of baseline vascular measurement categories to differentiate those whose visual acuity deteriorated from those who stayed stable or improved at 12 months, after controlling for other characteristics that were found to be significantly associated with the primary outcome in univariate analyses. Given the correlated nature of the data, the method of generalized estimating equations (GEE) was used to derive

ously included
d the outcome
dence intervals
ommercial soft-
ominal level of

regression coefficients. All covariates were simultane
into the model. Associations between a predictor an
were assessed using odds ratios (ORs) with 95% confi
(CIs). All statistical analyses were conducted using c
ware (SPSS version 18; SPSS, Inc., Chicago, IL). The n
statistical significance was set at 0.05.

TABLE 1. Characteristics of Eyes Treated with Intravitreal Ranibizumab Injections for Neovascular Age-Related Macular Degeneration over the 12-Month Study Period

Factor	Baseline	3 Months	6 Months	9 Months	12 Months
Vision (LogMAR)	0.73 (0.40)	0.60 (0.40)	0.66 (0.46)	0.71 (0.48)	0.70 (0.50)
CMT	332 (93)	230 (61)	247 (75)	244 (78)	237 (58)
Residual fluid, %	N/A	31.6	36.4	33.6	20.9
Number of injections	N/A	3.4 (0.6)	4.3 (1.6)	5.4 (2.1)	6.3 (2.8)
CRAE	146.03 (14.32)	147.27 (14.47)	147.43 (15.08)	145.99 (15.24)	146.36 (14.11)
CRVE	220.18 (25.77)	221.44 (23.31)	222.89 (23.33)	224.36 (24.05)	223.12 (21.68)

Values are expressed as mean (SD) unless otherwise noted. Residual fluid indicates percentage of eyes with residual intraretinal or subretinal fluid based on qualitative assessment of optical coherence tomography scan, not applicable (N/A).

(64.2%) and the mean age was 79.1 years (SD 7.9). Most lesions were classified as nonpredominantly classic (62.9%) and were generally 2 disc diameters or less in size (71.3%).

Table 1 shows the visual acuity and CMT changes through the 12-month follow-up period as well as the number of treatments given at each time point.

Despite the group level results (represented by mean scores) indicating no significant difference in visual acuity between baseline and final follow-up, a two-line change in vision occurred in several eyes over the study period, with 35% ($n = 31$) classified as having improved visual acuity and 20% ($n = 18$) classified as having worse visual acuity at 12 months compared with baseline.

Patient Characteristics Affecting Vision at 12 Months

The associations between patient/eye characteristics potentially influencing visual outcome at 12 months are summarized in Table 2. Sex, size of CNV lesion, and baseline CRVE were significantly associated with visual acuity outcome at 12 months ($P < 0.05$). Borderline associations were seen with baseline CMT, number of treatments given through to 12 months, and age of patient ($P < 0.10$). Eyes that deteriorated were found more frequently among males (56%) and had higher frequency of large CNV lesions at baseline (53%). After controlling for age, sex, and lesion size, baseline CMT, as well the number of treatments given, eyes that deteriorated had larger average venular caliber, 243.10 μm (95% CI, 227.01–259.19), than that of those who were stable, 214.30 μm (95% CI, 205.79–222.81), and those who improved, 215.26 μm (95% CI, 204.69–225.84; $P = 0.007$). Arteriolar caliber was not associated with visual outcome, with no significant difference between eyes losing vision, 150.12 μm (95% CI, 140.67–159.57), compared with that of those remaining stable, 143.64 μm (95% CI, 138.64–148.63) or gaining vision, 142.92 μm (95% CI, 136.71–149.13; $P = 0.69$). No correlation between the baseline CMT and baseline CRVE ($r^2 = 0.01$, $P = 0.33$) or CRAE ($r^2 = 0.01$, $P = 0.36$) was noted. Similarly, CMT change

TABLE 2. Associations between Baseline Characteristics and Change in Visual Acuity at 12 Months, in Eyes Treated with Intravitreal Ranibizumab Injections for Neovascular Age-Related Macular Degeneration

Factor	Better	Same	Worse	*P* Value
Sex, *n* (%)				0.03
Female	18 (58.1)	32 (78.0)	8 (44.4)	
Male	13 (41.9)	9 (22.0)	10 (55.6)	
Type of CNV, *n* (%)				0.06
NPC	15 (48.4)	31 (75.6)	10 (58.8)	
PC	16 (51.6)	10 (24.4)	7 (41.2)	
Size of CNV lesion, *n* (%)				0.02
≤2 disc areas	26 (86.7)	28 (68.3)	7 (46.7)	
>2 disc areas	4 (13.3)	13 (31.7)	8 (53.3)	
Hypertension, *n* (%)				0.62
No	9 (29.0)	16 (40.0)	6 (33.3)	
Yes	22 (71.0)	24 (60.0)	12 (66.7)	
Diabetes, *n* (%)				0.27
No	28 (93.3)	33 (82.5)	14 (77.8)	
Yes	2 (6.7)	7 (17.5)	4 (22.2)	
IHD/CVA, *n* (%)				0.94
No	22 (73.3)	30 (75.0)	14 (77.8)	
Yes	8 (26.7)	10 (25.0)	4 (22.2)	
Smoking, *n* (%)				0.16
Never smoked	6 (37.5)	8 (38.1)	6 (75.0)	
Past or current smoker	10 (62.5)	13 (61.9)	2 (25.0)	
Age, y, mean (SD)	75.65 (7.45)	78.59 (7.37)	80.72 (7.83)	0.06
Visual acuity at baseline, mean (SD)	0.81 (0.47)	0.70 (0.39)	0.60 (0.20)	0.17
CRAE at baseline, mean (SD)	144.32 (14.52)	143.90 (15.73)	148.23 (11.07)	0.44
CRVE at baseline, mean (SD)	223.96 (26.44)	219.04 (26.44)	245.02 (31.26)	0.01
CMT at baseline, mean (SD)	314.48 (74.92)	322.92 (104.31)	355.94 (77.37)	0.08
Number of injections at 12 mo, mean (SD)	7.16 (3.01)	5.73 (2.43)	5.94 (2.98)	0.09
Axial length, mm, mean (SD)	23.26 (0.66)	23.24 (0.83)	22.99 (0.98)	0.39
Spherical equivalent, D, mean (SD)	−0.08 (1.12)	0.53 (0.88)	−0.38 (2.93)	0.83

IHD, ischemic heart disease; CVA, cerebrovascular accident; NPC, nonpredominantly classic; PC, predominantly classic.

from baseline to 12 months was not associated with either CRVE change ($r^2 = 0.03$, $P = 0.20$) or CRAE change ($r^2 = 0.03$, $P = 0.18$). With respect to the treatment frequency, the mean number (range) of injections was 6.3 (3–13). In all, 19 eyes had four or fewer injections by 12 months. Of these the majority (11/19) were considered as "fluid-free" after the three injections and were found to have either improved or stable vision by 12 months, compared with baseline. A further 5 eyes were "fluid-free" after three injections and had no recurrence of intraretinal or subretinal fluid through 12 months. These eyes, however, lost vision due to progression of atrophy or subretinal fibrosis and were, in the opinion of the treating ophthalmologist, unlikely to benefit from further treatment. The final 3 eyes had recalcitrant fluid through the 12-month follow-up and continued to lose vision despite the initial "loading dose" injections. These eyes were found to have significant subretinal fibrosis and further treatment was also considered as unlikely to influence vision.

Table 3 shows a GEE model assessing the combined effects of patient/eye characteristics and their influence on visual outcome at 12 months. Characteristics found to have marginally significant associations with the outcome were also initially included in the model because their addition changed the regression coefficients by >5%. By combining CNV size and type, however, the regression model produced singularity, possibly due to insufficient cell numbers. Consequently, only CNV size was included in the model, given that it had the stronger initial association with the outcome.

After adjusting for age, sex, baseline CMT, and number of injections received, only CNV size and baseline CRVE were able to differentiate those whose vision deteriorated at 12 months from those who stayed stable or improved. Eyes that had larger CRVE at baseline and larger CNV lesions had significantly lower odds of their vision improving or staying the same at 12-month follow-up.

With respect to the 14 eyes without images at 12 months, the reasons for lack of data included: failure of attendance or transfer to other ophthalmologists (11 eyes of 11 patients) and death of patients (3 eyes of 3 patients).

No significant differences ($P > 0.05$) were observed in age (78.5 vs. 79.4 years), sex (64.9% vs. 60.6% females), baseline vision (0.74 vs. 0.73), baseline CRAE (144.6 μm vs. 147.8 μm), CRVE (218.8 μm vs. 221.9 μm), or CMT (325 μm vs. 348 μm) between the study completers and noncompleters.

Discussion

In this prospective study in eyes with active neovascular AMD treated with intravitreal ranibizumab injections, we found that baseline retinal venular caliber was a significant predictor of visual outcome at 12 months.

There are few studies that investigate the effect of ranibizumab on retinal vasculature.[18,19] These studies were of small sample size (11 eyes of 11 patients), and found a decrease in retinal arteriolar diameter at both the 3-month time interval and 1 year after the initiation of treatment. No assessment was made of retinal venular caliber or any potential influence on visual outcome.

Factors such as patient age, sex, size of CNV, and baseline vision have all previously been shown to influence visual outcome in patients with neovascular AMD.[20,21] In addition to these factors, we found that patients who lost two or more lines of vision at 12 months had significantly larger venular diameters at baseline compared with those who were stable or those who had significant visual improvement. This finding remained significant even after adjusting for age and sex (both of which have previously been shown to affect retinal vascular caliber[22]) as well as CNV size, baseline CMT, and number of injections given during the 12-month follow-up.

The specific underlying mechanism for our observations is unclear, but it is possible that the degree of retinal venular dilatation at diagnosis of CNV may be a marker of disease activity. In keeping with this hypothesis, retinal venular dilatation has been described as a predictive factor in the development of cardiovascular diseases such as stroke and coronary heart disease, independent of traditional cardiovascular risk factors.[23–26] Additionally, retinal venular widening has been demonstrated in primates injected with vascular endothelial growth factor.[27] As such, our observations of widening of retinal venules and poorer outcome of treatment with anti-VEGF treatment are interesting and may represent either more severe disease at baseline, with increased VEGF levels in the eye or, alternatively, a marker of end-stage disease, with neural disorganization or fibrosis, predicting poorer response to anti-VEGF therapy.

A weakness of our study was that only 80% of images were available at baseline at 12 months. We contend, however, that this is typical for this demographic of patients and no additional interventions were made (such as cataract surgery) purely to improve the data set. No significant differences were seen in CRAE, CRVE, and CMT in study eyes between those included in the analysis and those excluded at baseline. Accordingly, we feel that the general results were not substantially compromised. Another potential weakness of our study is the lack of standardized treatment protocol, with "as required" dosing allowed to maintain a "fluid-free" macula, rather than monthly dosing as seen in the pivotal randomized controlled

Table 3. Generalized Estimating Equations Modeling of the Effects of Baseline Characteristics on Visual Acuity Outcome at 12 Months in Eyes Treated with Intravitreal Ranibizumab for Neovascular Age-Related Macular Degeneration

	Same Compared with Worse			Better Compared with Worse		
Predictor	OR	(95% CI)	*P* Value	OR	(95% CI)	*P* Value
Central retinal vein equivalent at baseline	0.94	(0.90–0.99)	0.02	0.95	(0.90–0.99)	0.03
Sex						
Female	Reference					
Male	0.50	(0.07–1.04)	0.50	1.05	(0.11–9.54)	0.97
Age	0.90	(0.78–1.04)	0.16	0.85	(0.72–0.99)	0.04
Size of CNV lesion						
≤2 disc areas	Reference					
>2 disc areas	0.08	(0.01–0.59)	0.01	0.02	(0.00–0.28)	<0.001
Number of injections at 12 mo	1.02	(0.74–1.40)	0.92	1.18	(0.84–1.68)	0.34
Central macular thickness	1.00	(0.99–1.01)	0.50	0.99	(0.98–1.00)	0.25

Same compared with worse (CNV): chance of vision remaining within two lines of baseline compared with losing vision by two or more lines. Better compared with same: chance of vision improving by two or more lines compared with remaining within two lines of baseline.

trials. It is possible that monthly treatment may have influenced outcome, leading to better vision at 12 months. Finally, although the spherical equivalent of our patients did not differ significantly with respect to vision change at 12 months, it is possible that the axial magnification error due to refractive error may have influenced our results, although using a correction for refractive error based on the fundus camera (Zeiss [1 − 0.016] × spherical equivalent),[28] our results were unchanged, such that after controlling for age, sex, CNV size, baseline CMT, and number of injections, eyes that had significant deterioration in vision at 12 months still had a larger CRVE (234.44 μm; 95% CI, 218.32–250.56), compared with that of eyes that were stable (210.27 μm; 95% CI, 200.94–219.60) and those that improved (214.73 μm; 95% CI, 204.88–224.56; $P = 0.04$). This is consistent with our earlier work, indicating that the results are not affected by a potential bias due to axial magnification errors.

In summary, our prospective study shows that in eyes with neovascular AMD, baseline retinal venular caliber is an independent predictor of visual response at 12 months and may be related to the severity of disease.

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Clinical & Experimental Ophthalmology

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Editorial

Lessons learnt inform our approach to new antivascular endothelial growth factor treatments for neovascular age-related macular degeneration

Antivascular endothelial growth factor treatment (anti-VEGF) has revolutionized the treatment of neovascular age-related macular degeneration (nAMD). Ranibizumab (Novartis, Novartis Pharmaceuticals, Sydney, New South Wales, Australia), an affinity matured, humanized and monoclonal antibody fragment to VEGF,[1] was first approved for use in 2006. In 2007, it was listed on the Pharmaceutical Benefits Scheme in Australia, for subfoveal nAMD. This followed the results of the pivotal anti-VEGF antibody for the treatment of predominantly classic choroidal neovascularization (CNV) in AMD and minimally classic/occult trial of the anti-VEGF antibody ranibizumab in the treatment of neovascular AMD studies, which showed that monthly intravitreal ranibizumab was associated with a mean gain of +11.3 and +7.2 letters at 12 months ,and +10.7 letters and +6.6 letters at 24 months, in predominantly classic and minimally classic/occult lesions, respectively.

To reduce the treatment burden of monthly injections, many clinicians have used less frequent dosing strategies. During 2006–2007, treatment in the retina clinics at the Royal Victorian Eye and Ear Hospital (RVEEH), the first public hospital in Australia to offer this treatment, was most often to perform three monthly injections and then use a *pro re nata* treatment regimen. The decision to re-treat was based upon signs of CNV activity, presence of new haemorrhage, loss of visual acuity (VA) and optical coherence tomography findings (increase in central macular thickness or the presence of either intraretinal fluid or subretinal fluid). Four to six weekly follow up was maintained throughout this period.

An audit of the early results from RVEEH clinics analysed all patients with a diagnosis of subfoveal CNV secondary to AMD, who commenced intravitreal anti-VEGF treatment at the RVEEH from 2006 to 2007.

In all cases, patients were treated with ranibizumab, although rarely bevacizumab was given as a single 'one off' dose, as an initial treatment to avoid any treatment delay while obtaining approval for ranibizumab treatment. The VA outcome of patients who commenced treatment in 2006 and 2007 cohorts, with the mean ± standard deviation change in VA after 3, 6, 12 and 24 months, was +4.0 ± 11.9, +1.6 ± 12.6, −3.4 ± 15.5 and −6.9 ± 17.8 letters, respectively. These disappointing results were achieved with the mean number of 4.2 ± 2.0 injections in the first 12 months, an extremely low number of injections that, in part, reflects that some cases were chronic, where treatment was offered without any real expectation of improved outcomes. When no benefit was gained, the treatment was ceased after just a few months.

Industry-sponsored studies to determine if less frequent treatments would be as effective as monthly injections have reported similar, disappointing findings. The Efficacy and Safety of Ranibizumab in Subjects with Subfoveal CNV Secondary to AMD (EXCITE)[2] studies investigated the 12-month efficacy of quarterly dosing of ranibizumab after three consecutive monthly injections.[3] Both studies demonstrated that quarterly dosing regime was less effective than monthly treatment.

Other approaches being trialled that were based not upon fixed dosing schedules, but around the individual patient responses guiding the treatment frequency were more successful. Results from the small Prospective Optical Coherence Tomography Imaging of Patients with nAMD Treated with intraocular Ranibizumab study suggested that a flexible dosing regimen, with the optical coherence tomography-measured central retinal thickness and VA being used as guiding criteria for re-treatment, may be as effective as monthly treatment.[4] Similar results were also seen in the SUSTAIN study,[5] where treatment outcome was similar to the monthly pivotal trials, although with a lower average number of re-treatments (mean of 5.6 injections over 12 months). A small loss in VA, however, was seen after the initial gain from three monthly loading doses.

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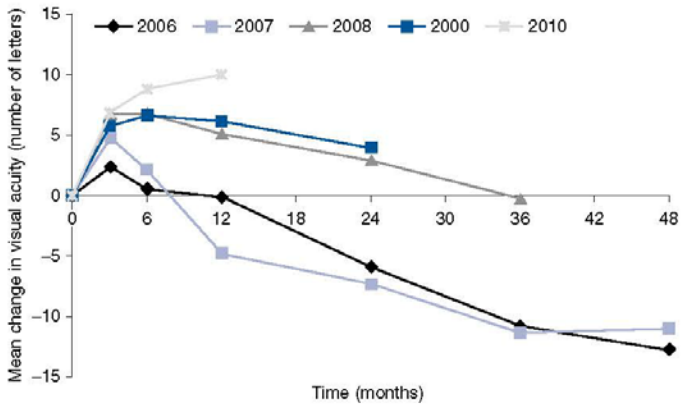


Figure 1. Vision change in patients treated with antivascular endothelial growth factor treatment (VEGF) injections for neovascular age related macular degeneration, characterized by year of treatment initiation. Number of treatments received: (a) 7.5 ± 2.1 injections over the first year. (b) 6.7 ± 2.5 and 4.4 ± 3.2 injections over the first and second years, respectively. (c) 5.1 ± 2.3, 4.4 ± 3.3 and 4.5 ± 3.1 injections over the first, second and third years, respectively. (d) 4.2 ± 2 injections over the first year, 2.2 ± 2.7 injections over the second year, 2.9 ± 3.3 injections over the third year and 3.1 ± 3.3 injections over the fourth year. (e) 3.9 ± 2.7 injections over the first year, 1.9 ± 2.2 injections over the second year, 1.2 ± 2.3 injections over the third year and 3.2 ± 2.6 injections over the fourth year. Number of letters indicates the number of ETDRS (Early treatment for diabetic retinopathy study) letters.

In VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD 1 and 2 studies, 2.0 mg/0.05 mL aflibercept dose, given monthly for three injections, followed by bi-monthly treatment gave equivalent visual outcomes to monthly (0.5 mg/0.05 mL) ranibizumab treatment at 1 year, with similar proportions of patients being prevented from losing 15 letters (95.3% *vs.* 94.4%) and gaining 15 letters of vision (31.0% *vs.* 32.4%), respectively. The mean gain in vision at 12 months was also similar, with approximately +8 letters gained in both groups.[9] This was achieved with 7.6 injections over 12 months.

It will be inevitable to wonder if the treatment interval for aflibercept could be extended beyond the eight weekly recommended injection frequency. However, we need to go cautiously and learn from the experience with ranibizumab where, in our endeavour to reduce the treatment burden, we lost visual benefit for the patient. It has become increasingly clear that a patient's requirements for anti-VEGF blockade varies, and we learnt from experience that long intervals might not be possible for all patients. While on average the treatment interval was longer in the VIEW studies with aflibercept, when compared to the ranibizumab interval in anti-VEGF antibody for the treatment of predominantly classic CNV in AMD and minimally classic/occult trial of the anti-VEGF antibody ranibizumab in the treatment of neovascular AMD, with similar VA results, it must be remembered that the results were averages, where some individuals did not have had the same good visual result with such long treatment intervals. Reasons behind these individual responses are not fully understood, but are likely to involve factors relating to the lesion type, individual characteristics and underlying genetic differences.[10–12]

Currently, no trial data exist to inform us if it is possible to go beyond the eight weekly intervals with aflibercept. Individualizing the treatment protocols seems logical rather than assuming that we can get all patients out to longer injection intervals. Let us learn from past experience. Clinicians who are just starting out to manage nAMD patients need to be cognizant of this history so that we do not go through the same learning process again, putting potential improvements in VA outcomes at risk by our desire to reduce the treatment burden.

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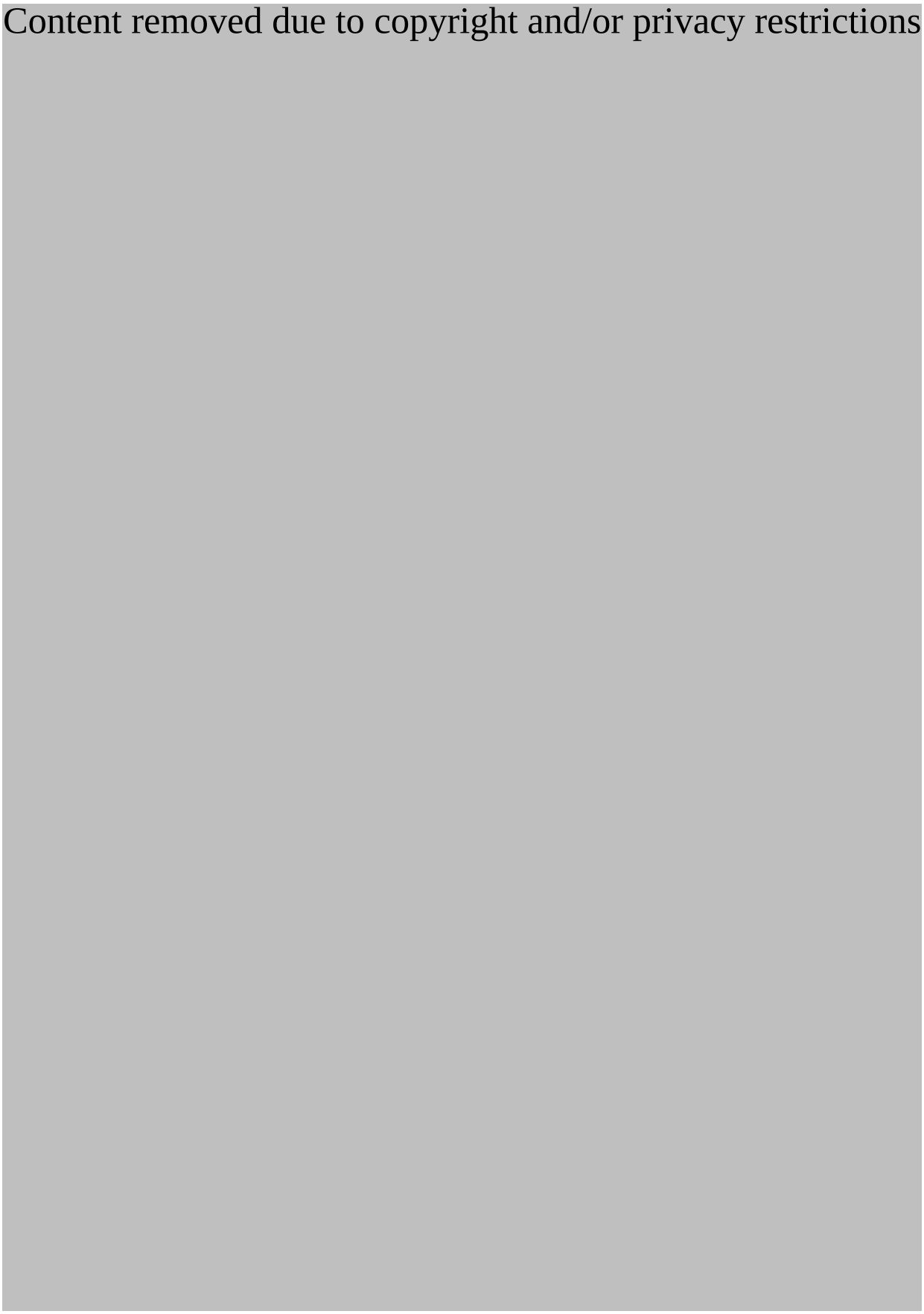
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Acute Intraocular Inflammation after Intravitreous Injections of Bevacizumab for Treatment of Neovascular Age-related Macular Degeneration

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Purpose: Bevacizumab is an inhibitor of vascular endothelial growth factor widely used as an "off-label" treatment of neovascular age-related macular degeneration (AMD), despite the lack of clinical trial data on efficacy or safety of this drug. We describe acute intraocular inflammation after intravitreous injection of bevacizumab for the treatment of neovascular AMD.

Design: A retrospective case series.

Participants: Patients with neovascular AMD treated with intravitreous injection of bevacizumab from clinical practices in 2 states (Victoria and South Australia) in Australia.

Methods: We retrospectively reviewed cases of acute intraocular inflammation after intravitreous injection of bevacizumab for the treatment of neovascular AMD.

Main Outcome Measures: The detection and description of inflammation in a large cohort of patients.

Results: There were 14 cases (11 women and 3 men), from a total of 1278 injections given. The mean age of patients was 83.7 years (range, 74–98). The majority had a prior injection of bevacizumab, with a mean number of injections of 2.7 (range, 1–6). Most patients presented within 24 hours of intravitreous injection, with rapid reduction in vision, but minimal discomfort. There were associated signs of ocular inflammation in the anterior and posterior segments of the eye. Visual acuity at presentation was substantially reduced compared with the preinjection acuity, although the vision rapidly improved with treatment over a period of 7–25 days toward preinjection visual acuity.

Conclusions: Intravitreous injection of bevacizumab for the treatment of neovascular AMD may be associated with acute intraocular inflammation. Differentiation from infectious endophthalmitis is important for appropriate management of this condition.

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Neovascular age-related macular degeneration (AMD) is the leading cause of visual loss in elderly persons ≥60 years. In the last few years, a new class of therapeutics based on suppression of vascular endothelial growth factor (VEGF) has been shown to be effective in preserving and even restoring vision in eyes with neovascular AMD.[1,2]

Bevacizumab (Avastin, Genentech, South San Francisco, CA) is a full-length anti-VEGF antibody of which ranibizumab is an affinity matured fragment. Bevacizumab has been widely used in an "off label" treatment for neovascular AMD because it is substantially cheaper than ranibizumab,[3] despite the lack of prospective randomized clinical trial data.[4] With regards to safety, several small case series have not found significant ocular or systemic morbidity with its use.[5–8] A recent international online 'survey' on the safety of bevacizumab noted only a very small minority of patients developed any complications after intravitreous injection, with no specific adverse event exceeding 0.21%.[9] A few case reports have reported mild anterior uveitis[10] and endophthalmitis[11] after injection.

We present a series of 19 cases of acute severe intraocular inflammation after intravitreous bevacizumab injection. To the best of our knowledge, such a large series has not been reported previously.

Methods

The 19 cases were seen from 6 clinical practices, in 2 states (Victoria and South Australia) in Australia over a period of 12 months (June 1, 2006 to May 31, 2007). At the Royal Victorian Eye and Ear Hospital, the study was approved by the Institu-

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tional Review Board and the data were collected as part of a prospective, uncontrolled, nonrandomized clinical trial. Ethics approval was not obtained from other sites. All sites conducted this study according to the tenets of the Declaration of Helsinki.

Bevacizumab was obtained from the manufacturer (Genentech) and processed with proper aseptic techniques to achieve the desired dosages according to strict guidelines[12] by accredited compounding pharmacies within the 2 states. After preparation, the bevacizumab syringes were transferred by courier to individual institutions in ice-filled Styrofoam boxes, without a temperature registering device. Before use, the syringes were placed in a refrigerator with monitored temperature between 2 and 8°C.

All participating retinal specialists followed well-established, standard protocols for intravitreous injections in a specifically designated "injection room" within the outpatient department. All intravitreous injections consisted of 1.25 mg/0.05 mL.[13]

After intravitreous injections, all patients were started on topical chloramphenicol 0.5%, 4 times a day for 3 days.

Results

Over a 12-month period, 1278 intravitreous injections of bevacizumab were given at 6 practices, within 2 states of Australia. Of these injections, 19 cases were associated with acute inflammation (1.49%).

Eight patients presented within a day of intravitreous injection, with rapid reduction in vision, but minimal discomfort. There were associated signs of moderate to severe ocular inflammation in the anterior and posterior segments of the eye.

Of the 19 patients, 5 had a very severe presentation, with marked vitritis and hypopyon in some cases. (Table 1 [available online at http://aaojournal.org]). Given the possibility of acute infectious endophthalmitis, anterior chamber and vitreous taps were performed. After vitreous tap, all these patients received intravitreous antibiotics. No microbial growth was observed in 2 aspirates, *Propionbacterium acnes* was found in 2 cases, and coagulase-negative staphylococcus species in 1 after 6 days of incubation in enrichment media. Independent specialist microbiological opinion determined these organisms as likely contaminants; however, given the possibility of infectious endophthalmitis in these cases, they have been excluded from further analysis.

As such, we present the clinical features of 14 patients with acute inflammation after intravitreous bevacizumab injection. Table 2 describes the demographics and clinical features of the 14 patients.

Of these patients, there were 11 women and 3 men. The mean ± standard deviation (SD) age of patients was 83.7±6.5 years (range, 74–98) and the mean (±SD) number of previous bevacizumab injections was 2.7±1.3 (range, 1–6). Visual acuity at presentation was substantially reduced compared with preinjection acuity, although vision rapidly improved with treatment over a period of 7–25 days toward preinjection visual acuity (Figure 1). The number of prior injections (Figure 2) did not seem to correlate with the degree of anterior chamber or vitreous inflammation. The time from injection to presentation with inflammation did not seem to affect the extent of visual recovery, although the time to recovery of vision was slightly longer in those patients presenting ≥2 days after injection (14.8±6.1 days compared with 10.8±3.3 days).

Case 1

A 90-year-old woman with an occult choroidal neovascular membrane had previously had 1 intravitreous injection of bevacizumab. Her vision had stabilized at 6/12, although there was continued evidence of angiographic leak and subretinal fluid and cysts on optical coherence tomography scan. She was injected uneventfully for a second time. Later that evening, she noticed worsening of her vision, although there was no pain or discomfort. On returning to the clinic 3 days later, her vision had worsened to 6/36 and there was a moderate degree of vitritis obscuring view of retinal details, with 1+ anterior chamber cells. No intravitreous or anterior chamber taps were done and she was treated with intensive topical steroids. One week later, her vitritis was clearing, although her acuity was still 6/36.

Case 2

An 82-year-old woman with a minimally classic choroidal neovascular membrane had previously had 1 uncomplicated intravitreous injection of bevacizumab. Before the second injection, her visual acuity was 6/48. Within 24 hours of injection, she noticed cloudy vision and on examination her vision had deteriorated to 1/60 and there were 2+ anterior chamber and 2+ vitreous cells. She was treated with intensive topical steroid therapy and by 14 days, her vision had improved to 6/60, with no evidence of residual inflammation.

Discussion

We report a series of patients with acute ocular inflammation associated with intravitreous bevacizumab injection from 6 clinical practices in 2 states within Australia.

The use of anti-VEGF agents in the treatment of AMD is regarded as an important breakthrough in the management of neovascular AMD. However, the long-term safety of intravitreous anti-VEGF drugs remains unclear. Phase III studies on ranibizumab demonstrated that between 1.3% and 2.2% of patients had moderate to severe inflammation after intravitreous injection.[1,2] The rate of inflammation seemed higher in patients receiving the 0.5-mg dose, although in the Minimally Classic/Occult trial of the Anti-VEGF Antibody, Ranibizumab in the treatment of Neovascular Age-Related Macular Degeneration trial, some degree of inflammation was also noted in 12.7% of patients in the sham injection group.[1] In these trials, most patients had minimal inflammation (defined by the number of cells and amount of protein in the anterior chamber and vitreous). It is not stated whether patients had visual symptoms or discomfort associated with the inflammation or whether they were asymptomatic and the inflammation discovered on scheduled examination.[1,2] As such, it is difficult to say whether this inflammation documented with ranibizumab is similar to that in our series.

Bevacizumab is derived from the same murine antibody as ranibizumab and has similar binding sites, but is a significantly larger molecule (165 kD) with potentially less effective retinal penetration and reduced affinity to bind VEGF. Unlike ranibizumab (which is an antibody fragment), bevacizumab has an Fc fragment, theoretically mak-

Table 2. Characteristics of Cases with Intraocular Inflammation after Bevacizumab Injection

Patient Details	Total No. of Injections	VA Before Last Injection	VA at Presentation	AC Activity	Vitreous Activity	Time from Injection to Presentation	Time Course of Visual Improvement and Final VA	Presenting Symptoms
1 85, M	2	0.30 (6/12)	0.70 (6/30)	1+	1+	Within 24 hrs	14 days 0.40 (6/15)	Reduced vision, floaters
2 82, F	6	0.70 (6/30)	1.00 (6/60)	1+	Haze	Within 24 hrs	14 days 1.00 (6/60)	Reduced vision
3 82, F	1	0.90 (6/48)	1.78 (1/60)	2+	2+	Within 24 hrs	14 days 1.00 (6/60)	Reduced vision, floaters
4 85, F	2	0.70 (6/30)	1.78 (1/60)	1+	1+	Within 24 hrs	7 days 0.70 (6/30)	Reduced vision, floaters
5 81, M	3	0.30 (6/12)	0.48 (6/18)	3+	2+	Within 24 hrs	7 days 0.30 (6/12)	Floaters
6 90, F	2	0.30 (6/12)	0.78 (6/36)	2+	2+	Within 24 hrs	11 days (0.78)	Reduced vision
7 86, M	4	0.60 (6/24-1)	1.48 (2/60)	2+	2+	Within 24 hrs	12 days 0.30 (6/12)	Reduced vision, photophobia
8 74, F	3	0.32 (6/12-1)	0.60 (6/24)	2–3+, keratic precipitates	Quiet	1 day	7 days 0.48 (6/18)	Reduced vision, photophobia
9 77, F	1	0.60 (6/24)	0.80 (6/36-1)	1+	1–2+ at posterior hyaloid face	2 days	19 days 0.52 (6/18+1)	Reduced vision, photophobia
10 93, F	3	1.00 (6/60)	1.30 (3/60)	1–2+	1+	2 days	13 days 0.74 (6/30-2)	Reduced vision, photophobia
11 98, F	3	1.00 (6/60)	1.48 (2/60)	1+	Quiet	3 days	11 days 1.48 (2/60)	Reduced vision
12 81, F	3	0.34 (6/12-2)	0.60 (6/24)	Nil	1–2+	6 days	13 days 0.30 (6/12)	Reduced vision
13 78, F	2	1.10 (6/75)	1.78 (1/60)	1+ keratic precipitates	Quiet	6 days	25 days 1.08 (5/60)	Reduced vision
14 80, F	3	0.78 (6/36)	1.30 (3/60)	1+	2+	1 wk	8 days 0.68 (6/30+1)	Reduced vision

AC = anterior chamber; F = female; M = male; VA = visual acuity (logarithm of minimum angle of resolution equivalent).
The AC and vitreous cells were graded using the Standardization of Uveitis Nomenclature classification.

ing the molecule more immunogenic. Bevacizumab is currently used as an "off-label" treatment for neovascular AMD in many countries; however, there are limited long-term safety data regarding ocular and systemic adverse reactions with its use. Several case series have found no serious ocular or systemic complications after intravitreous injection,[5–8] and a recently published international intravitreous bevacizumab safety survey[9] found similar experi-

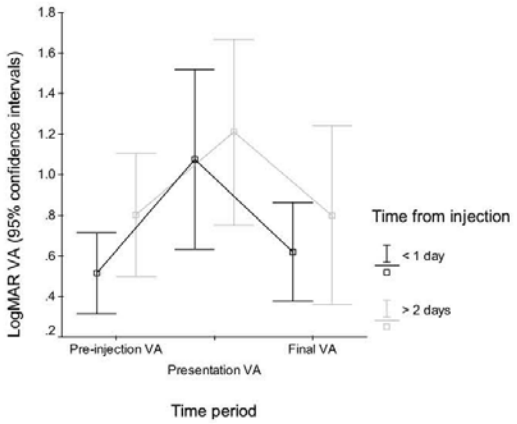


Figure 1. Graph showing the changes in visual acuity by time of presentation with symptoms. LogMAR = logarithm of the minimum angle of resolution; VA = visual acuity.

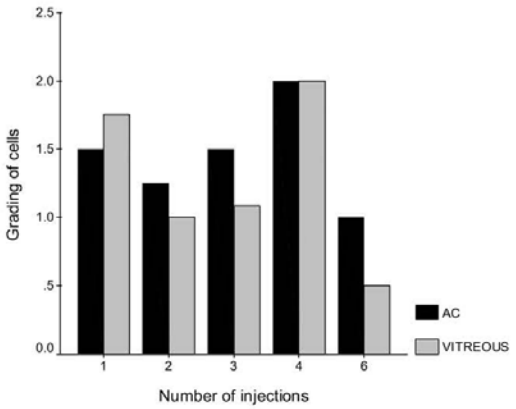


Figure 2. Bar chart showing the extent of anterior chamber (AC) and vitreous inflammation by the number of prior injections given (SUN Classification).

ences among retinal specialists from around the world. Apart from procedure-related adverse effects, there were minimal ocular and systemic events, which were thought to be an attributable to the drug. Ocular inflammation was documented in 0.14% of patients; most cases were graded as being mild to moderate and occurred between 2 and 7 days after injection. None of the cases had a hypopyon or progressed to endophthalmitis.[9] A similar rate of inflammation was also reported by the Pan-American Collaborative Retina Study group (0.09% of 1265 injections).[14]

The cases presented in our series of acute ocular inflammation associated with intravitreous bevacizumab had several factors in common, including the time of presentation, clinical features, and previous sensitization. The majority of patients had received prior injections of intravitreous bevacizumab and presented very soon after injection, with reduced vision and floaters. None of the patients had significant pain, although some admitted mild discomfort. Although many had some degree of vitreous inflammation, the majority had predominantly anterior chamber cellular activity. These features are atypical of infective endophthalmitis and suggest an inflammatory etiology to the process.

The underlying etiology of inflammation in this series is far from clear and we cannot be sure whether it relates to the bevacizumab molecule itself or to the way the drug was processed. Hence, we can only speculate as to possible mechanisms.

First, it is possible that contamination of the bevacizumab with bacterial endotoxin at the time of individual aliquot preparation may have occurred. After intravitreous injection, a reaction analogous to the toxic anterior segment syndrome after cataract surgery[15] may have taken place. Although theoretically possible, this scenario is unlikely; the incidence of this reaction seems to be sporadic and infrequent. It seems improbable that a bacterial endotoxin would be introduced at this stage of the bevacizumab preparation without widespread incidence of the inflammatory reaction. Second, bacterial endotoxin is a frequent and recalcitrant contaminant of antibody preparations during the production phase of the pharmaceutical.[16] Preparations of bevacizumab that are specifically prepared for intravenous use may contain traces of endotoxin at levels that incite intravitreous inflammation, even though they are of no significance when the drug is administered systemically. Third, the eye may itself mount an immune antiidiotypic response to the anti-VEGF antibody. It is interesting that the majority of cases of inflammation in our series had several previous injections of bevacizumab. The antiidiotypic immune response is mounted against an immunogenic molecule to which the eye has already been primed.[17] Larger molecules with Fc (constant) fragments as well as the antibody binding (Fab) fragments are more immunogenic than those with the antibody binding fragment alone. It is possible that with increased sensitization (after multiple injections), there may be an increased immune response. This would be in keeping with our series, in that most patients had prior injections of bevacizumab. Against this possibility, however, is that the degree of anterior chamber and vitreous inflammation did not increase with the number of prior injections given.

Fourth, storage of the drug may have been compromised. The manufacturer's guidelines state that bevacizumab vials must be refrigerated at 2 to 8°C (36 to 46°F), should be protected from light, and should be stored in the original carton until time of use. It is also recommended that bevacizumab is used within 8 hours of opening.[18] Whether any variation in storage temperature happened in our series is unknown, but theoretically some degradation of the drug may have occurred and rendered the injection more immunogenic. Temperature fluctuation has been demonstrated to increase the immunogenicity of therapeutic proteins.[19] This may be due to protein degradation creating novel antigenic epitopes not found in the parent molecule.[20]

In conclusion, we report clinical features of 14 patients with acute intraocular inflammation after intravitreous injection of bevacizumab. This series suggest that there is an underlying incidence of inflammation associated with the use of bevacizumab and is similar in frequency to that seen in using Ranibizumab in the Minimally Classic/Occult trial of the Anti-VEGF Antibody, Ranibizumab in the treatment of Neovascular Age-Related Macular Degeneration and Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in Age-Related Macular Degeneration trials. This inflammation has a particular clinical presentation and, although differentiation of severe inflammation from infectious endophthalmitis can be difficult, its early presentation and predominant anterior chamber involvement are specific clues.

We suggest that ophthalmologists continue to utilize compounding pharmacies adhering to the standards set out by the United States Pharmacopeia and be aware of the possibility of this clinical adverse effect when using bevacizumab for the treatment of neovascular AMD and other ocular conditions. We also advise a long-term safety and efficacy study examining the adverse effects of intravitreous bevacizumab.

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Table 1. Characteristics of Cases Excluded from Analysis, with Intraocular Inflammation after Bevacizumab Injection

Patient Details	Total No. of Injections	VA Before Last Injection	VA at Presentation	AC Activity	Vitreous Activity	Time from Injection to Presentation	Culture	Time Course of Visual Improvement and Final VA	Presenting Symptoms
1 79, M	4	0.48 (6/18)	Hand movements	4+ hypopyon	Haze	Within 24 hrs	*P acnes* from AC tap	7 days 0.70 (6/36)	Reduced vision
2 87, F	4	0.78 (6/36)	1.78	4+	No cells, slight haze	Within 24 hrs	Coagulase-negative *Staphylococci* from AC tap	7 days 0.78 (6/36)	Reduced vision, mild discomfort
3 84, F	2	0.48 (6/18)	Count fingers	3+	3+ hazy fundal view	Within 24 hrs	*P acnes* from AC tap	7 days 0.48 (6/18)	Reduced vision
4 82, F	3	0.18 (6/9)	1.48	4+	3+ retinal details visible	Within 24 hrs	No growth from AC/vitreous taps	14 days 0.26 (6/12+2)	Floaters, cloudy vision
19 82, F	2	0.60 (6/24)	Hand movements	3+ hypopyon	2+	3 days	No growth	21 days 0.78 (6/36)	Reduced vision

AC = anterior chamber; F = female; M = male; VA = visual acuity (logarithm of minimum angle of resolution equivalent); *P acnes* = *Propionobacterium acnes*.
AC and vitreous cells were graded using the Standardization of Uveitis Nomenclature classification.
All patients who had AC and vitreous taps received intravitreal injections of vancomycin 1 mg/0.1 mL, ceftazidime 2.25 mg/0.1 mL, dexamethasone 0.4 mg/0.1 mL. Intensive topical steroids and antibiotics were given after AC/vitreous tap.

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Survey of Ophthalmology

Major review

Predictors of anti-VEGF treatment response in neovascular age-related macular degeneration

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ABSTRACT

Currently available evidence on predictors of anti-vascular endothelial growth factor (VEGF) treatment response in neovascular age-related macular degeneration was reviewed. No meta-analysis of results is possible because of a lack of controlled and randomized trials, varying treatment regimes and outcome measures used, as well as suboptimal reporting. For genetic factors, most evidence to date has been generated for single nucleotide polymorphisms (SNPs) in the complement factor H (CFH), and VEGF-A genes. Just under half of the SNPs assessed in the CFH gene and 15% of the SNPs assessed in the VEGF gene were found to be associated with visual outcomes or the number of injections required during follow-up. Some evidence suggests association of worse treatment outcomes as well as a younger age at treatment onset with an increasing number of risk alleles in known risk genes (CFH and ARMS2/HTRA1) and polymorphisms in the VEGF-A gene. Clinical factors such as higher age, a better visual acuity (VA), a larger choroidal neovascularization (CNV) lesion at baseline, and a delay between symptom onset and initiation of treatment of more than 3 weeks also impact outcomes. Conversely, a worse acuity at baseline predicted more gain in vision. Overall, patients presenting with good acuity at baseline were more likely to have good VA at follow up, but the gain afforded by treatment was impacted by a ceiling effect. Most available evidence suggests a strong association of clinical factors such as age, baseline VA, and CNV lesion size with anti-VEGF treatment outcomes. No behavioral factors such as smoking influence treatment outcomes. Based on the studies conducted so far, the evidence suggests that underlying genotype of known AMD risk associated genes or of the VEGF-A gene have a limited effect, whereas presenting clinical factors appear to be more important in determining treatment outcomes.

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1. Introduction

Age-related macular degeneration (AMD) is the most common cause of blindness in all industrialized countries and affects a large proportion of the elderly.[5,25] Severe vision loss occurs in the late stages of the disease, classified into neovascular (nv) or "wet" AMD and atrophic or "dry" AMD, both stage IV disease according to the Age-Related Eye Disease Study classification.[6] Treatment that prevents a further deterioration of vision, and even an improvement in some, is only available for

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the late, neovascular stage of AMD.[93] Current treatment consists of regular intravitreal injections of anti-vascular endothelial growth factor (VEGF) agents ranibizumab (RBZ, Lucentis, Genentech, San Francisco, CA, approved use), aflibercept (Eylea, Regeneron, Tarrytown, New York, approved use) or bevacizumab (BVZ, Avastin, Roche, Basel, Switzerland, off-label).[96] Pegabtanip (Macugen, Pfizer, New York,) is another available drug for treatment of nvAMD, but not commonly used as it is clinically less effective than RBZ and BVZ.[87] RBZ and BVZ seem to be similarly effective in maintaining visual acuity over a 2-year period.[65] Although overall treatment response is good with an average gain of 7–15 ETDRS letters at 2 years of monthly treatment in phase III clinical trials, a certain proportion of patients (9–10%) continue to lose vision despite treatment. A second subgroup of patients initially regained lost vision and maintained that gain (30–38%), and a third subgroup slowly lost vision after an initial gain (50–60%).[14,15,81,82] The cut-off used for these subgroups in most studies is 15 ETDRS letters (gain or loss).[81]

1.1. *The importance of treatment response prediction*

The detrimental impact of impaired vision in AMD on vision-related quality of life (VRQoL) has been well documented using a number of instruments.[26] Vision impairment affects daily functioning, mobility, and emotional well-being.[63,78] Specifically, people with severe visual impairment have been shown to have an increased risk of falls,[41] depression,[13] life stress, and lower life satisfaction and activity levels.[12]

Blindness and visual impairment have cost implications, as does the treatment to avert it. In Australia, the annual direct medical cost caused by blindness and severe visual impairment was estimated at AUD$1.8 billion in the year 2004.[89] This cost places visual disorders seventh among diseases—ahead of coronary heart disease, diabetes, depression, and stroke—in terms of economic burden on the health system. Indirect costs were estimated to be double the direct costs at AUD$3.2 billion, making the total economic burden on Australian society in excess of AUD$5 billion.[89] An equally large amount, AUD$4.8 billion, was assigned to the loss of well-being (years of life lost as a result of disability and premature mortality).[18] A large proportion of this burden was due to AMD (20% of direct and 50% of indirect costs using the burden of disease–DALY methodology),[18] and in particular nvAMD.[89] These cost estimates, however, are drawn from data collected prior to 2004, when no anti-VEGF agents were available for the treatment of nvAMD. Direct costs can be expected to be much higher at present in Australia.[18] In the United States, the annual direct medical cost of all types of AMD was estimated to be US$575 million in 2004 when no anti-VEGF treatment was available.[79] A later estimation based on Medicare data calculated the total annual health care payments for Medicare beneficiaries who were newly diagnosed with nvAMD to be US$724 million in 2008.[23] This does not reflect the cost by patients with nvAMD who receive ongoing treatment (not newly diagnosed), nor any indirect cost caused by vision loss due to nvAMD. Overall costs caused by nvAMD can be expected to be much higher in the United States. Ranibizumab is currently in widespread use for neovascular AMD around the world. In Australia its monthly intraocular injection regime of AUS$2,000 per treatment is being fully subsidized by the pharmaceutical benefit scheme and repatriation pharmaceutical benefit scheme. Between 2008 and 2009 the pharmaceutical benefit scheme and repatriation pharmaceutical benefit scheme costs of this drug increased from AUS$108 million to $188 million in Australia. As the population is rapidly aging, the number of affected individuals will continue to substantially increase in future years, and treatment costs will continue to grow.

Gaining or at least maintaining vision in a large proportion of treated cases results in a positive effect of anti-VEGF treatment on a patient's VRQoL in nvAMD.[11,20] In order to optimize treatment outcomes as well as the use of available resources and minimize cost, treatment outcome prediction based on factors assessable at presentation is of major importance. As with most new therapies, the population of patients treated as well as treatment outcomes in real life differ considerably from the large phase III clinical trials. This makes frequent reassessments of treatment strategies and optimal treatment for various patient subgroups in standard practice necessary as new information emerges. In order to aid such re-assessments, we collated all available and relevant information on factors assessable at baseline that might have predictive value in relation to anti-VEGF treatment outcome in nvAMD.

2. Predictive factors

The majority of patients (80–90%) maintain vision with anti-VEGF treatment, with approximately 30% of these showing a significant improvement. Despite uniform treatment of all patients, however, 10–15% do not show any treatment benefit and continue to lose vision. Another group gains initially, but subsequently loses vision, either rapidly, because of adverse events such as subretinal hemorrhage, or in a prolonged fashion, as the result for example of the development of atrophy. To date, it is unclear why this happens. Several factors have been suggested as either causes or proxies, such as age, choroidal neovascularization (CNV) lesion characteristics at baseline, and several genetic factors, but the number of studies trying to shed light on this is still somewhat limited. Relevant published data, and factors thought to influence treatment outcomes, hereafter referred to as Predictors of Outcome, are summarized in the following sections.

2.1. *Genetic factors*

Several genes such as CFH, HTRA1/ARMS2, C3, CFB/C2, and APOE have been shown to be associated with increased risk of developing AMD.[19] Currently available literature regarding the influence of these genetic polymorphisms in the response to anti-VEGF treatment, however, is inconsistent (see Table 1).

2.1.1. *Complement Factor H gene*

Most studies to date have investigated the Complement Factor H (CFH) gene with conflicting results being reported (Tables 1 and 2). A number of studies have reported an association with visual acuity outcomes at 1 and 3 months,[38,91] 6 months,[66,71] 9 months,[10,55] and at 12 months[27,49] follow-up; others, however, failed to identify any association with

Table 1 – Summary of studies assessing predictors of outcome for anti-VEGF treatment in Neovascular AMD

Study	n	Treatment and follow-up	Predictors	Outcome
Genetic predictors of outcome				
Abedi et al[1,2] Australia	224 FU at 12/12	RBZ or BVZ	CFH ARMS2 HTRA1 C3 C2/BF CFHR F13 B VEGFA	BCVA improvement at 3/12, 6/12, and 12/12: • HTRA1-rs11200638 AA associated with larger gain (no. of letters)[1] • AMRS2-rs3793917 CC associated with better and TT with worse outcomes (no. of letters)[1] • Rs 302500 VEGFA TT or TC associated with +7 letters compared to CC genotype at 6/12 (n – 201)[2]
Boltz et al[7] Austria	141 (185 eyes)	BVZ FU between 42 – 1182 days	VEGF	BCVA at the end of treatment: • VEGF-rs3024997 GG and VEGF-rs2010963 GG associated with worse BCVA in univariate analyses • No associations in multivariate analyses
Bozorg et al[9] USA	264	BVZ and/or RBZ FU ?	CFH C5 C3 C2	BCVA improvement ≥ 1 line or CRT decrease: • No associations
Brantley et al[10] USA	86	BVZ, PRN at 6/52 intervals Mean FU 9/12	CFH ARMS2	BCVA change at mean FU of 9/12: • CFH Y402 H CC associated with less improvement
Francis et al[28] USA	65	RBZ FU at 12/12	GWAS	BCVA (change in ETDRS letters) at 6/12 and 12/12: • CFH associated with less improvement CRT change at 6 and 12/12: • C3 associated with reduced CRT Persistent leakage on FA at 6/12 and 12/12: • FLT1 associated with persistent leakage Number of RBZ injections at 12/12: • VEGFA, FLT1, CFH associated with fewer injections
Franklin et al[29] USA	66	RBZ or BVZ FU at 12/12	ARMS2	Mean BCVA change at 12/12: • ARMS2 insertion/deletion associated with improvement, ancestral genotype associated with deterioration
Hagstrom[33] et al (CATT study) USA	834	RBZ or BVZ Monthly or PRN FU at 12/12	CFH ARMS2 HTRA1 C3	No associations found with: • Mean VA at 12/12, mean change in VA, 15 letter or more increase in VA • Retinal thickness, mean change in foveal thickness, fluid on OCT • Leakage on FA • Mean change in lesion size • Mean number of injections

(continued on next page)

Table 1 – (continued)

Study	n	Treatment and follow-up	Predictors	Outcome
Imai et al[40] Japan	83 (23 with PCV)	BVZ, regimen not specified FU at 1/12 and 3/12	CFH HTRA1 VEGF PEDF	BCVA at 1/12 and 3/12: • CT genotype in CFH0-rs1061170 predicts worse BCVA outcomes at 1/12 and 3/12 • VEGF-rs699947 associated at 1/12 (nature of association not reported) • PEDF-rs1136287 associated at 3/12 (nature of association not reported) CRT at 1/12 and 3/12: • No associations CNV recurrence at 3/12 and 6/13: • No associations
Inglehearn et al[41] UK	202	RBZ (upload + PRN) FU at 6/12	CFH ARMS2 HTRA1 VEGF	BCVA a improvement (>5 letters) or quantitative trait: • No associations
Kang et al[47] Korea	75	2.5 mg BVZ (Upload + PRN) FU ≥ 6/12	CFH ARMS2 HTRA1	BCVA at 6/12: • ARMS2-rs10490924 high risk allele (TT) associated with larger improvement
Kitchens et al[50] USA	100	RBZ or BVZ FU ?	CFH ARMS2 VEGF	Response classified as indefinite response, delayed relapse, immediate relapse and never responded: • ARMS2 TT genotype associated with classification as never responded
Kloeckener-Gruissem et al[51,52] Switzerland	215[51] 238[52]	RBZ and BVZ (some with upload, most PRN only) FU at 12/12 and 24/12	CFH CFB HTRA1 ARMS2 VEGFA FZD4	BCVA at 12/12[51]: • CFH-rs1061170 CC associated with less improvement (OR 3.42) • Heterozygosity at CFH-rs1061170 and FZDa-rs10898563 associated with being a "good responder" (OR 3.66) BCVA at 24/12[52]: • CFH-rs1061170 CC associated with less improvement (OR 3.58)
Kovach et al[54] USA	52	BVZ and/or RBZ FU ≥ 12/12	CFH ARMS2	Proportion of responders (BCVA ≥2 lines, CRT decreased): • Response associated with having at least one risk allele in CFH and ARMS2
Lee et al[57] USA	156	RBZ, PRN only FU ≥ 9/12	CFH	No. of injections at FU ≥ 9/12: • CFH Y402 H TT associated with fewer injections, TC (OR 1.25) and CC (OR 1.37) with more injections
McKibbin et al[68] UK (Caucasian)	104	RBZ (Upload + PRN) FU at 6/12	CFH VEGF HTRA1	BCVA change proportion >5 ETDRS letters gain: • CFH-rs1061170 TT smallest proportion who gain BCVA change in absolute ETDRS letters: • HTRA1-rs11200638 GG worst gain
Nakata et al[72] Japan	173	94 with BVZ (PRN ± loading phase) 79 with triple therapy (BVZ, PDT and 2 mg iv triamcinolone) FU at 12/12	VEGF	Mean BCVA change at 12/12: • VEGF-rs699946 AA associated with loss, GG associated with most gain, irrespective of BVZ or triple therapy

Nischler et al[73] Austria	197	BVZ every 6/52 until macula dry FU at least 6/12	CFH 402	Proportion who lost ≥3 lines in distance and reading BCVA: • CFH402 HH: 41% and 46% (P − 0.04 and P − 0.02) • CFH402 YY: 28% and 26% • CFH402 YH: 26% and 24%
Noh et al[74] Korea	146	RBZ (Upload) FU after upload (3 monthly injections)	CFH ARMS2 HTRA1 VEGFA VEGF-R (KDR)	BCVA improvement and CRT improvement at 4/12: • No associations
Orlin et al[78] USA	150	RBZ and/or BVZ FU mean 22/12–24/12	CFH ARMS2 HTRA1	Response/Non-response at 22/12–24/12: • No associations with response (Y/N), no. of injections or absoclute change in BCVA
Park et al[79] Korea	280	RBZ (upload + PRN) FU at 5/12	23 SNPs in 14 genes, genes not specified	Proportion with BCVA ≥2 lines and FA improvement at 5/12: • VEGFA-rs699947 associated with good response (OR 2.85; P − 0.022 unadjusted for multiple testing)
Smailhodzic et al[87] Caucasian	397	RBZ FU at 4/12, after 3 monthly injections	CFH ARMS2 VEGFA KDR LPR5 FZD4	BCVA (ETDRS letters) at 4/12: Carrier of all four high risk alleles in CFH and ARMS2, and risk alleles in VEGF • have a mean 10 letter loss (versus patients without risk alleles) • are younger at begin of treatment (65.9 vs 75.3 years in patients with no risk alleles)
Teper et al[92] Poland	90	RBZ as PRN FU at 12/12	CFH ARMS2	BCVA mean change at 12/12: • ARMS2 A69s homozygosity associated with poor response
Tian et al[93] China	144 (no PCV)	BVZ at 6/52 intervals FU at 3/12	CFH ARMS2 HTRA1 VEGF SERPING1 C3	BCVA change (ETDRS letters) at 3/12: • CFH-rs800292 CC worst outcomes (compared to CT and TT; P − 0.034) • ARMS2-rs10490924 TT worst outcomes (compared to TG and GG; P − 0.026) • HTRA1-rs11200638 AA worst outcomes (compared to GA and GG; P − 0.022) CRT change at 3/12: • No associations
Tomita et al[95] Japan	50 (30 with PCV)	RBZ (upload + PRN) FU at 12/12	CFH ARMS2	BCVA at 3/12 and 12/12 and CRT at 3/12: • No associations
Wang et al[97] USA	93	RBZ or BVZ FU at 6/12	CFH HTRA1 IL-17 IL-23 R CYP3 A LEP VEGFA	BCVA and CRT improvement as proportion of responders (maintain or improve BCVA and decrease CRT): • IL-23R-rs10127763 was associated with response (higher frequency of allele A in non-responders)
Wickremasinghe et al[98] Australia	192	RBZ and/or BVZ (75% had loading phase, then PRN) FU at 12/12	APOE	BCVA at 3/12: • APOE ε4 allele (compared to ε2 allele) associated with better outcome (OR 4.00)
Yamashiro et al[97] Japan	105 (56 with PCV), 78 genotyped	RBZ (loading phase + PRN) FU at 12/12	CHF ARMS2	BCVA changes at 12/12: • No association

(continued on next page)

Table 1 – (continued)

Study	n	Treatment and follow-up	Predictors	Outcome
Clinical predictors of outcome				
Boyer et al[8] (MARINA 2 yr results)	478	238 RBZ 0.3 mg monthly 240 RBZ 0.5 mg monthly FU at 24/12	Age Baseline BCVA CNV size	Mean BCVA (ETDRS letters) and proportion loosing/gaining ≥15 letters: • Higher age • Larger CNV size • Better baseline BCVA Associated with less gain in ETDRS letters
Guber et al[31]	182	RBZ (PRN) Mean FU 1.3 years	OCT features Age Baseline BCVA	CRT Response on OCT: • CME associated with larger CRT reduction than spongoid type ME, subretinal fluid or PED • No additional gain after 6 injections • Age and baseline BCVA no influence on CRT response
Heimes et al[35]	128	RBZ (Upload + PRN) FU at 12/12	Baseline BCVA CNV type FAF Retinal sensitivity	Mean BCVA at 12/12: • Worse baseline BCVA associated with larger gain, better baseline BCVA with less gain • Classic CNVs gain more • Eye with abnormal foveolar FAF gain less • Eye with low central retinal sensitivity (<4 dB) gain less CAVE: No multivariate testing!
Heimes et al[36]	95	RBZ or BVZ ± PDT, regimen not specified FU at 5/12	FAF	Mean BCVA improvement: • Abnormal central FAF associated with less gain CAVE: No multivariate testing!
Horster et al[39]	28	RBZ and/or BVZ as PRN with 3 monthly injections in cases of recurrences FU 12 – 43/12	CNV type	Recurrences: • All classic membranes had at least two recurrences during FU • Occult membranes had recurrence only in 61% of cases CAVE: No multivariate testing!
Introini et al[42]	125 with vascularized PEDs	38 PDT 44 iv triamcinolone with PDT 50 BVZ or RBZ Mean FU 20/12	Baseline BCVA CNV (+vascularized PED)	Risk of RPE tear: • better baseline BCVA • CNV (rather than RAP lesion) Associated with higher risk CAVE: No multivariate testing!
Jeng et al[44]	72 eyes	RBZ or BVZ FU ≥ 12/12	OCT features	No. of injections during FU: • No OCT features (PED–size or fluid, sub-retinal fluid, CME, sub-retinal tissue) at baseline were associated
Jyothi et al[45]	Pooled analysis	BVZ FU at 3/6 and 6/6	Prior treatment Baseline BCVA CNV characteristics OCT features	Mean change in BCVA at 6/12: • No prior treatment • Better baseline BCVA • Predominantly classic membranes Associated with more gain • Baseline CRT not associated
Kaiser et al[46] (ANCHOR 1 yr results)	280	140 RBZ 0.3 mg monthly 140 RBZ 0.5 mg monthly FU at 12/12	Baseline BCVA CNV size Age	Mean BCVA (ETDRS letters) and proportion losing/gaining ≥15 letters: • Lower baseline BCVA, • smaller CNV size, • lower age associated with more gain ETDRS letters

Kang et al[48]	64	RBZ PRN only FU at 12/12	Baseline BCVA Baseline CNV size	BCVA change at 12/12: • Better baseline VA predicts higher risk to loose VA at 12/12 • Larger CNV diameter associated with worse VA at 12/12
Lalwani et al[56] PrONTO	37	RBZ (Upload + PRN) FU at 24/12	Baseline BCVA CNV characteristics	Mean no. of re-injections: • No association with baseline BCVA or lesion characteristics or size.
Lee et al[59]	148	RBZ or BVZ (Upload + PRN) FU ≥ 12/12	Vitreomacular adhesion	Mean BCVA: • Presence of vitreomacular adhesion associated with less gain in BCVA CAVE: No multivariate testing!
Lim et al[63]	185	RBZ (Upload + PRN) FU at 6/12	Delay between symptom onset and fist treatment Age Baseline BCVA	Proportion with 0.2 logMAR change (gain/lose) or no change: • Longer delay (>21 days) • Higher age (≥85 years) • Better baseline BCVA Associated with less gain
Lux et al[64]	43	BVZ (1 + PRN) FU at 6/12	Baseline BCVA Baseline reading ability CNV characteristics	Response classified as improvement in BCVA or reading ability: • Baseline reading ability was lower and CNV size larger in non-responders (stable or worse) CAVE: No multivariate testing!
Mariani et al[66]	99	RBZ (Upload + PRN) Mean FU 21/12	PED	Secondary loss of BCVA—mean change between 3/12 and end of FU: • Presence of PED at baseline was associated with secondary loss of BCVA
Mettu et al[70] Lad et al[55]	38 with serous PEDs[70] 33 with classic CNV[55]	RBZ or BVZ (induction with 3-5 monthly injections) FU after induction	ICG lesion characteristics	Persistence of activity (on OCT or FA): • Arteriolarized vascular complex (AVC) • Polypoidal choroidal vasculopathy (PCV) On ICG associated with persistent activity in serous PEDs[70] • Capillary subtype (much more than mixed or AVC subtype or RAP) Associated with resolution of activity[55]
Tannan et al[90]	185	Anti-VEGF, regimen not specified Mean FU 20/12–29/12	OCT features	Need for continuous anti-VEGF treatment: • Presence of subretinal fluid on OCT associated with need of continuous anti-VEGF treatment
Tolentino et al[94]	568	Pegaptanib (every 6 weeks) after induction (1–3 injections) with other anti-VEGF agent FU at 11/12	Baseline BCVA OCT features	Mean BCVA: • Worse baseline BCVA • OCT feature: presence of foveal pit, absence of central fibrovascular tissue, normal IS/OS and preserved photoreceptor layer Associated with more gain
Wickremasinghe et al[98]	192	RBZ or BVZ (75% had loading phase, then PRN) FU at 12/12	Age Baseline BCVA	BCVA at 12/12: • Younger age associated with improvement (OR 0.94) • Better baseline BCVA associated with less improvement (OR 3.24)
Yamashiro et al[97]	105 (56 with PCV),	RBZ (loading phase + PRN) FU 12/12	Age CNV lesion diameter	BCVA changes at 12/12 in non PCV cases only: • Higher age and greater linear CNV diameter predicted worse outcome
Ying et al[99] CATT study	1107	RBZ or BVZ, monthly or as PRN FU at 12/12	Age Baseline BCVA OCT features CNV characteristics Presence of GA IOP	BCVA gain at 12/12: • Older age, better baseline BCVA, larger CNV, predominantly and minimally classic lesions, absence of RAP lesion, presence of GA, abnormally high IOP, thicker total fovea thickness and presence of RPE elevation on OCT Associated with less gain

(continued on next page)

Table 1 – (continued)

Study	n	Treatment and follow-up	Predictors	Outcome
Behavioral predictors of outcome				
Inglehearn et al[41] UK	202	RBZ (upload + PRN) FU at 6/12	Smoking	BCVA a improvement (>5 letters) or quantitative trait: • History of smoking (current or ex) associated with positive outcome
Kovach et al[54] USA	52	BVZ and/or RBZ FU ≥ 12/12	Smoking	Proportion of responders (BCVA ≥2 lines, CRT decreased): • No association
Menger et al[69]	100	BVZ and or RBZ FU 24/12	Smoking Current use of antihypertensives	Mean BCVA: • Increasing no. of pack years (current or former smoker) • Current use of antihypertensives Associated with lower BCVA

FU, follow-up; BVZ, bevacizumab; RBZ, ranibizumab; BCVA, best corrected visual acuity; CRT, central retinal thickness; PCV, polypoidal choroidal vasculopathy; CFH, complement factor H; HTRA1, high-temperature requirement A-1; VEGF, vascular endothelial growth factor; PEDF, pigment epithelium-derived factor; PRN, Pro re nata; SERPING1, serpin peptidase inhibitor; clade G (C1 inhibitor), member 1; C3, complement component 3; iv, intravitreal; PDT, photodynamic therapy; GWAS, genome wide association study; FLT1, fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor); APOE, Apolipoprotein E gene; OR, odds ratio; CFB, complement factor B gene; FZD4, frizzled family receptor 4; OCT, optical coherence tomography; CME, cystoids macular edema; ME, macular edema; RAP, retinal angiomatous proliferation.

treatment.[39,76,90,97] Within the studies that report an association, several have found the CC risk genotype of single nucleotide polymorphism (SNP) rs1061170 (Y402 H) in the CFH gene to be associated with a less favorable outcome of treatment as well as a need for more injections during follow-up.[10,49,50,55] Contrary to this, one study reported the CT genotype of this SNP to be associated with worst outcome.[38] The AG genotypes of the SNP rs194918455 has been association with more injections during the first year of treatment with RBZ compared with the AA genotype.[27] Conversely, the TT protective genotype was found in one study to be the least likely to gain vision (>5 ETDRS letters).[66] Thus both risk and protective genotypes have been associated with worse outcome in these studies. The largest study to date did not find any association with mean change in acuity, several optical coherence tomography (OCT) parameters, leakage on fluorescein angiography (FA), mean lesion size, or mean number of injections at 1 year.[32]

2.1.2. *Age-related maculopathy susceptibility 2 (ARMS2)/ high-temperature requirement A-1 genes (HTRA1)*

In the case of the ARMS2 gene, homozygosity for the TT genotype at SNP rs10490924 (A69 S) increases the risk of losing vision or not improving under treatment.[90] Similarly, the TT genotype is associated with a lack of response to treatment[48,91] and the CC genotype with better treatment outcomes.[1] The T-allele at SNP rs10490924 confers a higher risk to develop AMD.[80] In addition, the AA homozygous genotype at SNP rs11200638 in the high-temperature requirement A-1 (HTRA1) gene,[24] which is associated with an increased risk to develop AMD, has beeen associated with a larger gain in vision[1] with other studies reporting the opposite.[91] Interestingly, some studies report better treatment outcomes for patients with AMD risk alleles in these genes.[28,45,52] Other studies, however, could not find an association between ARMS2/HTRA1 and treatment outcomes.[32,39,49,50,97]

2.1.3. *Vascular endothelial growth factor gene*

The human VEGF-A gene consists of nine alternatively spliced exons that regulate interactions with HSPGs and neuropilins.[56] Most cells produce several VEGF-A isoforms simultaneously, with 121 and 165 being predominant.[75] VEGF production can be induced in cells that are not receiving enough oxygen.[36] Oxygen-deficient cells produce hypoxia-inducible factor (HIF), a transcription factor. HIF stimulates the release of VEGF, among other functions (including modulation of erythropoeisis). Circulating VEGF then binds to VEGF receptors on endothelial cells, triggering a tyrosine kinase pathway leading to angiogenesis.[36] As highlighted earlier, current standard treatment for nvAMD is based on an anti-VEGF approach.

A number of studies have assessed the association between the VEGF gene and anti-VEGF treatment outcomes in nvAMD, using a large number of different SNPs (Table 2). The majority of studies did not find an association,[2,39,48,49,50,66,72,91,94] or only an association in univariate analyses that was no longer present when controlling for other factors such as age and baseline best-corrected visual acuity (BCVA).[7] An American study on white patients reported

an association with fewer injections[27] and one Korean and two Japanese[38,70,77] studies reported associations with BCVA results, which could not be reproduced in another Korean study.[72] As these studies investigated ethnically different samples, it is impossible to compare directly the results as gene variants may vary substantially between ethnicities.[17]

2.1.4. *Apolipoprotein E gene*

A number of studies have demonstrated a higher risk of development of AMD with the apolipoprotein E (APOE) ε2 polymorphisms compared with the ε3 and ε4 variants.[95] In particular the ε4 variant may be protective as it may aid in the response to oxidative stress and facilitate greater lipid efflux from retinal pigment epithelium (RPE) cells and across Bruch's membrane.[95] In one study, the presence of the protective APOE gene ε4 allele was associated with improved treatment outcomes compared to those with an ε2 allele, after controlling for baseline acuity, treatment delay from first symptoms, as well as age and sex.[95]

2.1.5. *Complement system related genes*

Several studies assessed the influence of genes involved in the complement system. No specific associations with polymorphisms in the complement factor two (C2), three (C3), five (C5), B (CFB), or complement factor H receptor (CFHR) gene in determining treatment outcome have been noted.[1,9,32,49,50,91] Only one study found C3 to be associated with reduced central retinal thickness (CRT) on OCT following treatment.[27]

2.1.6. *Other genes*

Polymorphisms in the C-reactive protein gene may be associated with worse visual outcome following anti-VEGF treatment.[10] An interaction between the SNP rs1061170 in the CFH gene and the SNP rs10896563 in the frizzled family receptor 4 gene (which encodes a transmembrane protein involved in the development and/or maintenance of retinal vasculature[98]) in cases who were heterozygous at both tested SNPs has been reported to predict good response at 12 months[49] but was no longer present at 24 months.[50] One study assessed the interleukin (IL) 17 and 23 genes, and found the IL-23 R gene A allele to be associated with a higher risk of non response (less visual gain and no CRT decrease).[94] There are a number of other genes implicated in AMD pathogenesis, such as the complement factor I gene, genes of enzymes involved in the metabolism of cholesterol other than Apo-E, and the tissue inhibitor of metalloproteinase 3 gene.[31] To date none of these genes has been associated with anti-VEGF treatment outcomes in nvAMD. Similarly, adverse treatment outcomes such as RPE atrophy or subretinal hemorrhage have not been associated with any of the known AMD risk genes.

2.1.7. *Genome-wide association study*

One genome-wide association study (GWAS) on 65 patients found an association of the AA genotype in the SNP rs 1065489 of the CFH gene with less improvement, and the AA genotype in the SNP rs 6660704 in the C3 gene with a reduced CRT on OCT at 12 months follow-up. The AG genotype of the SNP rs 3766404 in the CFH gene and the AG genotypes in the SNP rs 833068 and the SNP rs 833069 in the VEGFA gene predicted a higher number of injections needed during follow up (12 months) compared with the AA (CFH), or AA/GG (VEGF) genotypes, respectively.[27] The GWAS identified one additional gene, the fms-related tyrosine kinase 1 gene (FLT1), which is a receptor to VEGF, to be associated with persistent leakage on FA and fewer injections over 12 months.[27]

The largest pharmacogenetic study (n = 397) to date assessed several AMD risk alleles in known risk genes (CFH, AMS2/HTRA1) and polymorphisms in the VEGF gene in relation to treatment outcomes and age at onset of treatment.[85] With an increasing number of risk alleles, treatment outcomes were worse and age at onset of treatment lower. Cases with all four high-risk alleles and newly identified risk alleles in the VEGF gene had a mean 20 letter loss from baseline to follow-up after loading and were on average 10 years younger at onset of treatment (70 vs 80 years) compared with cases with no risk alleles.[85]

In summary, genetic studies report contradicting associations for risk as well as protective alleles in known AMD susceptibility genes. A number of additional genes such as the VEGF, FZD4, or APOE gene, may be causally related to the anti-VEGF treatment approach in nvAMD and have been associated with vision outcomes, anatomical outcomes, or the number of injections required during follow-up. The evidence available to date does not explain treatment outcomes through pharmacogenetic associations, and other factors, such as clinical or behavioral factors, may be more important.

2.2. *Clinical factors*

The majority of studies reported factors predictive of final treatment outcomes, with most studies assessing vision outcomes, followed by anatomical outcomes, adverse events, and number of injections required during follow-up. The factors or predictors associated with functional outcomes were commonly measured as an absolute or relative improvement in best corrected distance vision. Factors associated with anatomical outcomes were most often measured as change in CRT on OCT or response on angiography, and factors associated with adverse events were measured as risk of, or occurrence of RPE rips, hemorrhage, etc. Factors associated are discussed with the number of injections required during follow-up separately.

2.2.1. *Predictors of vision outcomes*

There is ample evidence generated in the pivotal phase III clinical trials (e.g., ANCHOR, MARINA, PrONTO, CATT) that increasing age, poorer visual acuity, and larger CNV lesion at presentation predispose to worse treatment outcomes, and that better baseline visual acuity is associated with a higher risk of losing vision.[8,44,81] This is also reflected by a large number of studies assessing treatment outcomes in clinical practice outside of these trials.[43,46,61,92,95,97,99]

Several studies have reported a negative influence of previous treatment such as photodynamic therapy or intravitreal triamcinolone on achievable functional outcomes.[43,59,62] An abnormally high intraocular pressure may be associated with less gain in vision.[99]

Different CNV lesion types—occult, minimally classic, and predominantly classic on FA—have been reported to respond differently to anti-VEGF treatment. Predominantly and

Table 2 – Associations reported for SNPs assessed in various genes in relation to anti-VEGF treatment response in neovascular AMD

Gene	Abedi et al[1,2]	Boltz et al[7]	Brantley et al[10]	Francis et al[28] GWAS	Hagstrom et al[33]	Imai et al[40]	Inglehearn et al[41]	Kloeckener-Gruissem et al[51]
CFH: SNPs tested 19x, association found 9x: 9/19 = 0.47								
Rs800292						NF		
Rs1061170			VA at 9/12		NF	VA at 1/12 and 3/12	NF	VA at 12/12
Rs1065489				VA at 12/12				
Rs3766404				No. of injections at 12/12				
Rs10801555								
Rs1410996						NF		
ARMS2:2/7 = 0.29								
Rs10490924			NF		NF			NF
Rs3750848								
Intergenic region								
Rs3793917								
HTRA1: 2/7=0.29								
Rs11200638					NF	NF	NF	NF
Rs932275								
VEGF: 4/27 = 0.15								
Rs699946								
Rs699947		NF				VA at 1/12		
Rs833068				No. of injections at 12/12				
Rs833069		NF		No. of injections at 12/12				
Rs1005230		NF						
Rs1413711		NF					NF	NF
Rs1570360						NF		
Rs2010963		NF				NF		
Rs3024994	NF							
Rs3025000	+7 letters at 6/12							
Rs3025010	NF							
Rs3025030	NF							
Rs3025033								
Rs3025035	NF							
Rs3025039		NF						
Rs3025042	NF							
Rs3025047	NF							
PEDF: ¼ = 0.25								
Rs12150053						NF		
Rs12948385						NF		
Rs9913583						NF		
Rs1136287						Predicting VA at 3/12		
SERPING1: 0								
Rs1005510								
Rs2511989								
C3: 1/3 = 0.33								
Rs2230199					NF			
Rs2230205				CRT at 6/12 and 12/12				
Rs2250656								
CFB								
Rs641153								NF
FZD: 1/1 = 1								
Rs10898563								Response at 12/12 in combination with CFH

FU, follow-up; NF, none found; CRT, central retinal thickness; VA, visual acuity.
This table includes only studies that reported which SNPs were assessed.

Table 2 – (continued)

Lee et al[57]	McKibbin et al[68]	Nakata et al[72]	Nischler et al[73]	Orlin et al[78]	Teper et al[92]	Tian et al[93]	Wickrema-singhe et al[98]	Yamashiro et al[97]
						VA at 3/12		NF
Injections over >9/12	VA at 6/12		VA at FU > 6/12	NF	NF	NF		NF
						NF		
						NF		
				NF	VA at 12/12	VA at 3/12		NF
				NF				
				NF				
	VA at 6/12			NF		VA at 3/12		
				NF				
		VA at 12/12						
		NF						
						NF		
	NF							
		NF						
		NF						
						NF		
						NF		
						NF		
						NF		
						NF		

minimally classic lesions were associated with less gain in vision[99] and more frequent recurrences requiring treatment[37] compared with occult lesions. This association, however, was not confirmed in several other studies.[54,62] Conversely, some studies reported classic CNVs (predominantly and/or minimally) to gain more vision.[34,43] One study reported those eyes with retinal angiomatous proliferations (RAP) gained more vision than eyes with other lesions.[99]

Several OCT characteristics have been associated with functional treatment outcomes. The presence of a foveal pit, absence of central fibrovascular tissue, a normal inner segment/outer segment, and photoreceptor layer on OCT led to more gain in vision.[92] Interestingly, CRT—a global measure—at baseline was not related to functional outcomes at 6 months,[43] whereas foveal thickness and presence of a RPE elevation were found to be associated with less gain in vision at 12 months.[99] Signs of anatomical integrity of the retina, such as presence of a continuous external limiting membrane or inner segment/outer segment band were associated with better visual acuity outcomes after more than 6 months;[73] they were a much weaker predictor than baseline visual acuity, however.[73] Contrary to these studies, Kolb et al did not find any OCT baseline parameters to be predictors of 12-month visual outcomes.[51]

Recently, there has been increasing interest in the vitality of the RPE and photoreceptor cell layer as a predictor of anti-VEGF treatment outcomes in nvAMD. In a large, pooled analysis of participants with a clinically meaningful loss or gain of vision (≥15 ETDRS letters) in two phase III clinical trials (ANCHOR and MARINA), vision loss after 2 years of treatment was associated with signs of RPE dysfunction such as pigmentary abnormalities, atrophic scars, and absence of leakage on FA.[81] In two other smaller studies (n = 95 and n = 128), RPE abnormalities, as seen on fundus autofluorescence were the only factors independently associated with better treatment outcomes.[34,35] These two studies, however, did not control for confounders in multivariate testing.

A pigment epithelial detachment (PED) at baseline may confer a higher risk of secondary loss of vision at follow-up after three monthly injections of RBZ.[64] The presence of vitreomacular traction or adhesion reduced the chance of visual improvement, but the study did not control for confounders.[57]

Another factor associated with outcomes is the interval between onset of symptoms and presentation and treatment. Lim et al found a delay of more than 21 days between symptom onset and treatment predicted a higher risk to lose or not gain vision at 12 months follow-up.[61]

2.2.2. *Predictors of anatomical outcomes*

On OCT, a cystoid type edema (i.e., intraretinal cystic changes present on OCT) has been found to be associated with larger reduction in CRT than other types of macular edema, subretinal fluid, or a PED.[30] In another study those with a cystoid macular edema or a thick layer of subretinal tissue present on OCT had a higher risk of nonresponse at 12 months follow-up.[16] No additional reduction in CRT was observed after the six initial injections in one study and age and baseline BCVA was not associated with CRT response to treatment.[30]

On indocyanine green angiography, an arteriolarized vascular complex or a polypoidal choroidal vasculopathy in serous PEDs predicted persistent lesion activity,[68] whereas a capillary subtype in classic membranes was predictive of a resolution of activity[53] after an induction period (3–5 monthly anti-VEGF injections).

2.2.3. *Predictors of adverse events*

One study assessed the influence of factors at baseline on subsequent development of a rip of the RPE in cases with vascularized PEDs and found better baseline BCVA and the presence of a CNV rather than a RAP lesion conferred a higher risk to develop a RPE rip.[40] Again, these results were not adjusted for possible confounders. No other studies reported predictors of adverse events such as the occurrence of subretinal hemorrhage or atrophy.

2.2.4. *Predictors of required injections*

Predominantly and minimally classic lesions were associated with less gain in vision[99] and more frequent recurrences requiring treatment.[37] The presence of subretinal fluid on OCT at baseline was associated with a need for ongoing treatment during the first 20 months.[88] Another study did not find any OCT features to be associated with the number of injections required over the first year of treatment, however.[42] Interestingly, the PrONTO study did not find any associations between the mean number of re-injections over 2 years and baseline BCVA, CNV lesion characteristics, or lesion size.[54] As the majority of studies only report number of injections, and rarely intervals between injections, it remains unclear as to what predictors are associated with the possibility to extend a patient's retreatment interval.

2.3. *Behavioral factors*

There has been little study of the effect of known behavioral risk factors such as smoking and lifestyle on anti-VEGF treatment outcomes. Menger et al reported an increasing number of pack years, both for current and former smokers, resulted in less gain in vision at 2 years follow up,[67] whereas Inglehearn found those with a history of smoking (current and former) gained in vision.[39] Others have found no relationship between smoking and treatment outcomes.[52] Those on antihypertensives gained less in vision at 2 years.[67] The use of anticoagulants in patients with nvAMD undergoing anti-VEGF treatment has not been assessed in large studies. A small series of six cases found four out of six patients (66%) who developed a macular hemorrhage after stabilization with anti-VEGF treatment to be on anticoagulants (1 warfarin, 1 aspirin, 1 clopidogrel, 1 aspirin and warfarin).[58] Kiernan et al found that anti-coagulant use may predispose patients with nvAMD to intraocular hemorrhage more so than age and duration of disease alone.[47] The effect of concurrent anti-VEGF treatment was not addressed, however.

3. Evaluation of evidence

Available data stem from studies that widely vary in their study population, size, and quality of study design. The vast majority are observational case series, often retrospective, which report factors found to be associated with a range of

outcomes. Few randomized, controlled clinical trials beyond the initial phase III clinical trials for RBZ and now, more recently, the randomized, controlled RBZ and BVZ head-to-head trials have reported predictors of outcomes.

3.1. Outcome measures

Main outcome measures are most often distance visual acuity, either a mean change, a mean difference, or a proportion of gainers, maintainers, or losers. Mean change or mean difference are often based on different measures (ETDRS or Snellen charts) reported as letters or logMAR scores. Categorization ranges from two (responders/nonresponders), three (e.g., gain/maintain/lose), to four or five (e.g., gain & maintain/gain & early loss/gain & gradual loss/no gain/immediate loss) with varying thresholds (e.g., change in logMAR score by >0.2 or ETDRS letters >5). Less often, anatomical outcome measures, mostly CRT as assessed by OCT, other OCT or FA features, or occurrence of adverse events, are used.

Distance visual acuity as an outcome measure comes with a number of challenges related to its inherent measurement error. Under conditions likely to minimize variability, a significant change in visual acuity requires about ±0.14 log units change to reach 95% confidence.[4] As 0.1 log units change corresponds to 5 letters or a line on a letter chart, this threshold is about 8 letters or 1.5 lines, conservatively, before a measured change in visual acuity can safely be assumed to be an effect rather than a measurement error. Against this background, a reported gain in vision with treatment may actually be small or nonexistent in a number of studies, as cut-offs are often set at 1 line or 5 letters. In addition to this methodological challenge, these outcomes are assessed at different times in most studies, as early as after the first injection to as late as after several years (Table 1). A number of studies did not adequately control for known potential confounders, such as baseline age, visual acuity, or lesion size.

The Comparison of AMD Treatment Trials (CATT) study highlighted that there may be a difference between BVZ and RBZ in regards to the risk of developing atrophic changes.[65] Because the CATT study was not powered to explore adverse events such as this, further studies are needed.

3.2. Study populations

All studies comprise heterogeneous samples, with varying subtypes of AMD (including RAP and polypoidal choroidal vasculopathy [PCV]), who receive the same treatment (RBZ, BVZ, or pegaptanib) but according to various treatment regimes (1 + PRN, upload + PRN, monthly, combination treatment). In studies conducted in Asia, mostly Korea and Japan, there is a much higher incidence of AMD subtypes such as PCV that are rare in samples of white patients.[22] Similarly, the genetic makeup of Asian samples is quite different, and several AMD risk genes have been found to have different associations with development of early- and late-stage AMD compared with studies based on samples of white patients.[29] Again, this has implications for the generalization of findings to other populations, in particular in regards to the reported genetic associations (Table 1).

3.3. Collation of data

No collation of data using mean effects or mean change was done, as data are too diverse, and the standards of studies and their reporting are not adequate to allow this. For example, in order to create Forrest plots, commonly used in meta-analyses, assessment of predictors of outcomes and also effect modifiers should be considered when identifying true prognostic factors. In addition, studies need to be at least controlled, ideally randomized, and need to report the same treatment outcomes (pre and post mean, difference, odds ratios, proportions) with regard to all prognostic factors identified.[60,69] Unfortunately, this is not the case for the vast majority of studies, which is why we have opted only to summarize available data descriptively.

3.4. Summary of data

In summary, several factors, in particular age, visual acuity at baseline, CNV lesion size, and delay to treatment, have been implicated in treatment response to anti-VEGF in nvAMD. For these factors, most available evidence suggests a strong association with anti-VEGF treatment outcomes—with higher age, a better VA, a larger CNV lesion at baseline, and a delay between symptom onset, and initiation of treatment of more than 3 weeks predicting a higher risk to lose or gain less vision. Conversely, a lower VA at baseline was associated with a higher risk to gain vision. Overall, patients presenting with good acuity at baseline are more likely to have good acuity at follow-up, but will not have gained significantly with treatment as the result of a ceiling effect. Evidence regarding CNV characteristics on FA and OCT features present at baseline is more controversial, with a number of studies reporting some association with visual outcomes or number of injections. Other studies, however, failed to reproduce this. Evidence regarding the influence of smoking (measured as pack years or current/ex-smoking) exemplifies the current dilemma, with one study finding smoking to be associated with worse, and another study with better, treatment outcomes, whereas another did not find any association (Table 1).

Genetic predictors of outcome have been assessed in a large number of trials, with most evidence being presented for the CFH and VEGF genes. Of all the studies reviewed, just under half of the SNPs in the CFH gene reported were found to be associated with VA outcomes or the number of injections required during follow-up (Table 2). For the VEGF gene, 15% of assessed SNPs were found to be associated with visual acuity outcomes or the number of injections required during follow-up. Both outcomes (VA or number of injections) were assessed at varying time points and in populations differing in race (white vs Asian). Only data from one of the large, randomized controlled trials regarding pharmacogenetic associations in anti-VEGF treatment for nvAMD has been reported, and most available data stem from uncontrolled, prospective, or retrospective case series. The largest pharmacogenetic study to date has reported worse treatment outcomes as well as a younger age at treatment onset with an increasing number of risk alleles in known risk genes (CFH, AMS2/HTRA1) and polymorphisms in the VEGF gene.[85]

Based on the identified studies, the effect of known genetic risk variants assessed with treatment outcomes is weak at best,[29] and clinical factors at presentation (baseline) seem to be more important in determining treatment outcomes.

4. Need for further research

A number of questions associated with anti-VEGF treatment of nvAMD remain to be clarified, and can only be addressed in well-designed, large studies. Currently, there is no alternative to anti-VEGF treatments available. As a result, studies will have to remain uncontrolled for the time being as it is unethical to not treat patients presenting with nvAMD, which will continue to create challenges for the assessment of prognostic factors of treatment outcomes. Larger, more uniform studies, however, would at least allow for a synthesis of results and thus a better understanding of the influence of various factors assessable at baseline that may predict treatment outcomes.

Our review led us to conclude that studies must more clearly define different subtypes of AMD such as PCV, RAP, and AMD itself, and use a common protocol to assess treatment outcomes or at least comparable outcome measures. They need to optimize and unify treatment regimes as much as possible, and improve the quality of reporting.

There will be various special circumstances, such as anti-VEGF treatment in the presence of atrophy, extensive subretinal hemorrhage, or a poor presenting VA in the affected eye.[3] These will likely never be sufficiently addressed by larger studies, and treatment decisions must be based on the little evidence available as well as expert opinion and previous experience. Similarly, it has to be assessed whether patients presenting for treatment at centers involved in studies are representative of the population at large. Oishi et al have demonstrated that poor baseline VA is a risk factor for patient-directed cessation of treatment.[74] We did not find that any of the reviewed studies accounted for this potential bias.

As outlined herein, utilized outcome measures should not only be comparable, but also sensitive and reliable. Beatty et al have advocated the use of reading speed and mesopic contrast sensitivity as being more sensitive outcome measures than BCVA for patients undergoing anti-VEGF treatment, in particular in eyes where baseline BCVA is logMAR 0.7 or better.[84] Similarly, measuring solely distance VA might underestimate the impact of vision impairment on everyday life. Rubin et al found VA, contrast and glare sensitivity, stereoacuity, and visual fields to be significant and independent risk factors for self-reported visual disability in an elderly population and concluded that VA alone was not the only dimension of the association with subjective disability.[83] Against this background, measuring only distance VA as an outcome measure and using cut-offs within the range of measurement error may not do justice to the ultimate beneficiary of this intervention—the patient.

Although several anti-VEGF therapies are available, including aflibercept, ranibizumab (RBZ), bevacizumab (BVZ), and pegaptanib, few data are available directly comparing these different anti-VEGF agents. Only one, the Comparison of AMD Treatments Trial (CATT), has directly compared RBZ and BVZ, and published data on the influence of several AMD susceptibility genes.[32] The CATT trial reported a very similar efficacy of both anti-VEGF agents and failed to find any influence of the investigated genes on anti-VEGF treatment outcomes in the subsample (834 out of 1,149 participants) assessed.[32] The VIEW studies have directly compared RBZ and aflibercept, and found aflibercept to be non-inferior to RBZ.[33] These findings were published only recently and further details are yet to come. As more anti-VEGF agents and combination treatments become available, the pharmacogenetics should be compared to determine whether a certain subgroup of patients responded better to a certain drug based on their genetic profile.

Similarly, to date little evidence is available regarding the influence of factors that can be influenced by the individual clinician, such as treatment protocol (i.e., when to extend or stop monthly injections and start PRN), experience with these new drugs, and the delay between presentation and initiation of treatment. Currently, available evidence is insufficient in regards to the interaction between drug type, treatment regime, and genetic, clinical, and behavioral factors.

5. Conclusion

Predictors of treatment outcomes are necessary to not only plan treatment in the most cost-effective way and inform the patient about his or her individual prognosis, but can also be used to select the most appropriate treatment and treatment protocol. With regard to the latter, however, a relative difference in anti-VEGF treatment response associated with genotype or clinical characteristics is of little relevance when all treatment choices available are based on the anti-VEGF approach, and there is no alternative treatment.[29] This may change, and a combination of genetic and clinical factors may help in deciding for the best treatment out of several options for an individual patient.

Current treatment regimes, however, can be modified as to the frequency of injections and/or follow-up, which may be beneficial for a subpopulation of patients at high risk of loss of vision who, for example, are older, have a larger lesion size, or have a better visual acuity at presentation. Similarly, the amount of anti-VEGF drug injected may be increased in patients who did not respond well to standard injections of 0.5 mg RBZ.[21] Initial results of the small study in which poorly responding patients were injected with 2 mg RBZ monthly are promising, showing a better functional and anatomical outcome.[21] In treating naive patients a higher dose of RBZ does not seem to confer any additional benefit.[86]

In conclusion, a number of clinical factors, namely, age, baseline VA, and lesion size, predict anti-VEGF treatment outcomes, and favor those who present earlier and thus are younger, have smaller CNV lesions, and have a better baseline VA; this will lead to better final VA, but not a larger gain in those with good baseline VA due to a ceiling effect. Genetic factors may be associated with anti-VEGF treatment outcomes, whereby a larger number of risk alleles in known

AMD risk genes predict worse treatment outcomes and earlier onset, although the effect of these known genetic factors currently appears to be small. With subsequent, larger, and thus better-powered studies, the size and nature of an influence of genetic factors on treatment outcomes in nvAMD may become more evident. Other genes so far not identified may predict outcome better, but this will require a genome-wide pharmacogenetic approach to identify them.

6. Methods of literature search

Medline, PubMed, Web of Science, and ARVO Abstracts were used to search the literature from 2006 to 13 April 2012. Supplemental sources including references contained in identified articles were used. Findings were limited to English and German articles and English abstracts of foreign language articles. All animal studies were excluded. Key words searched were: ("age-related macular degeneration" OR "AMD" OR "macular degeneration" OR "maculopathy" OR "drusen" OR "choroidal neovascularization") AND ("anti-VEGF treatment" OR "anti-VEGF" OR " bevacizumab" OR "ranibizumab" OR "pagabtanib" OR "Lucentis" OR "Avastin" OR "Macugen")

For genetic factors:

AND

("risk gene" OR "CFH gene" OR "ApoE gene" OR " HTRA gene" OR "LOC gene" OR "ARMS2 gene" OR "VEGF gene" OR "PEDF gene")

For clinical factors:

AND

("delay*" OR "metamorphopsia" OR "CNV" OR "lesion" OR "lesion size" OR "lesion characteristics" OR "OCT" OR "leakage" OR "hemorrhage" OR "bleeding" OR "macular edema" OR "PED" OR "epithelial detachment" OR "family history" OR "second eye" OR "age" OR "gender" OR "prior treatment" OR "race" OR "Caucasian" OR "African" OR "Asian" OR "Hispanic")

For behavioral factors:

AND

("smoking" OR "nutrition" OR "alcohol drinking" OR "BMI" OR "diet" OR "fish" OR "fruit" OR "vegetables" OR "antioxidants" OR "copper" OR "lutein" OR "zeaxanthin" OR "carotenoids" OR "trace elements" OR "trace minerals" OR "zinc" OR "vitamin*" OR "supplement" OR "selenium")

Articles were excluded if they were reviews, case reports, case series of fewer than 10 patients, or if design and/or reporting were of a standard that did not allow for inclusion. For example, studies that did not adjust for factors known to influence treatment outcomes such as age, baseline VA, and CNV lesion size or CNV lesion characteristics in multivariate models were only included if they reported an interesting finding which was not reported by another, better designed and conducted study.

7. Disclosures

No authors have any conflict of interest related to this article.

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